A1CX3

cobas®

Tina-quant Hemoglobin A1cDx Gen.3

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056668 190	Tina-quant Hemoglobin A1cDx Gen.3 (500 tests)	System-ID 2066 001	**cobas c** 503
08445699 190	Tina-quant Hemoglobin A1cDx Gen.3 (200 tests)	System-ID 2066 002	
04528417 190	Calibrator f.a.s. HbA1c (3 × 2 mL)	Code 20674	
05479207 190	PreciControl HbA1c norm (4 × 1 mL)	Codes 20001-20004	
05912504 190	PreciControl HbA1c path (4 × 1 mL)	Codes 20011-20014	
08463107 190	A1CD (Hemolyzing Reagent) (50 mL)	System-ID 2069 001	
08463093 190	SCCS (Special Cell Cleaning Solution) (50 mL)	System-ID 2905 001	
11488457 122	HbA1c Hemolyzing Reagent for Tina-quant HbA1c (1000 mL)	For Hemolysate Application only	

English

For use in the USA only

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

This method is certified by the National Glycohemoglobin Standardization Program (NGSP).

This device has significant negative interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF including those with known Hereditary Persistence of Fetal Hemoglobin. Refer to the Limitations - interference section of this method sheet for details.

Intended use

The Tina-quant Hemoglobin A1cDx Gen.3 assay is intended for use as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes. It is an in vitro diagnostics reagent system intended for quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in hemolysate or venous whole blood on the **cobas c** 503 clinical chemistry analyzer. HbA1c determinations are useful for monitoring of long-term blood glucose control in individuals with diabetes mellitus.

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

Hemoglobin (Hb) consists of four protein subunits, each containing a heme moiety, and is the red-pigmented protein located in the erythrocytes. Its main function is the transport of oxygen and carbon dioxide in blood. Each Hb molecule is able to bind four oxygen molecules. Hb consists of a variety of subfractions and derivatives. Among this heterogeneous group of hemoglobins HbA1c is one of the glycated hemoglobins, a subfraction formed by the attachment of various sugars to the Hb molecule. HbA1c is formed in two 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.

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. Glucose levels closer to the time of the assay have a greater influence on the HbA1c level.[1]

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.[4,5]

For monitoring long term glycemic control, testing every 3 to 4 months is generally sufficient. In certain clinical situations, such as gestational diabetes, or after a major change in therapy, it may be useful to measure HbA1c in 2 to 4 week intervals.[7]

Test principle[9,10,11]

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.[12,13]

*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):[14]

HbA1c (%) = (HbA1c/Hb) × 91.5 + 2.15

Reagents - working solutions

R1 Antibody Reagent

MES[a] buffer: 0.025 mol/L; TRIS[b] 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.

A1CD (Hemolyzing Reagent, Cat. No. 08463107190)

Aqueous buffered matrix, pH 7.25; tetradecyltrimethylammonium bromide: 36 g/L; sodium dihydrogenphosphate monohydrate: 16 mmol/L; sodium monohydrogenphosphate dihydrate: 64 mmol/L; stabilizers; preservatives

a) MES = 2-morpholinoethane sulfonic acid

b) TRIS = Tris(hydroxymethyl)aminoethane

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

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

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

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

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

Reagent handling

Ready for use

Storage and stability

A1CX3

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.

A1CD (Hemolyzing Reagent)

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.

When storing at temperatures under 3 °C, the reagent may become cloudy. This has no effect on the function of the reagent and is reversible at higher temperatures. It is therefore recommended to equilibrate the reagent at room temperature for approximately 10 minutes and mix thoroughly before use.

On-board in use and refrigerated on the analyzer: 4 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. Anticoagulated venous blood or hemolysate.

The only acceptable anticoagulants are Li-heparin, K_2-EDTA, K_3-EDTA, Na-Heparin, EDTA-fluoride 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 –20 °C (± 5 °C)

The recovery of HbA1c ratio values from sedimented samples, especially in case of poorly controlled diabetic patients, may be slightly elevated. To minimize this effect, samples may be gently mixed by inversion prior to analysis.

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 –20 °C (± 5 °C)

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

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

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

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

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 ▪ after 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.[15,16] Results can be transferred to results traceable to DCCT/NGSP using the published master equation and following the recommendation of 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 the International Diabetes Federation (IDF).[14,17,18,19]

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[12,13,20,21,22,23,24,25,26,27]

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. Glycated HbF is not detected 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).[13,28]
4. 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.[25] 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.
5. Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with a hemoglobinopathy but normal red cell turnover (e.g. sickle cell trait).
6. 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.
7. Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with hereditary spherocytosis, malignancies or severe chronic hepatic and renal disease.
8. Hemoglobin A1c should not be used to diagnose diabetes during pregnancy. It reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival.
9. mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) are not suitable for the diagnosis of gestational diabetes.[29]
10. In 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.[29]
11. Hemoglobin A1c testing should not replace glucose testing for type 1 diabetes, in pediatric patients and in pregnant women.

Criterion: Recovery within ± 7 % of initial value.

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

Lipemia (Intralipid):[24] No significant interference up to an Intralipid concentration of 400 mg/dL. There is poor correlation between triglycerides concentration and turbidity.

Triglycerides: No significant interference up to a triglycerides concentration of 1584 mg/dL.

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

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

Total protein: No significant interference up to 21 g/dL protein spiked into the sample.

Drugs: Tests were performed on 18 drugs. No significant interference was found at the concentrations tested.

Drug	Concentration (mg/dL)
Acetaminophen	20
N-Acetyl-L-Cysteine	166
Acetylsalicylic acid	100
Ampicillin-Na-salt	100
Ascorbic acid	30
Na-Cefoxitin	660
Cyclosporine A	0.5
Doxycyclin	5
Gammagard	6000
Heparin	5000 IU/L
Ibuprofen	50
Levodopa	2
Methyl-L-Dopa	2.25
Metronidazole	20
Phenylbutazone	40
Rifampicin	6
Theophylline	10
Tolbutamide	300

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.

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 information. For further instructions refer to the operator's manual.

Limits and ranges

Measuring range

Hemoglobin: 4-40 g/dL (2.48-24.8 mmol/L)

HbA1c: 0.3-2.6 g/dL (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 13.2 g/dL (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 = 0.50 g/dL (0.31 mmol/L)

Limit of Detection = 1.00 g/dL (0.62 mmol/L)

HbA1c:

Limit of Blank = 0.19 g/dL (0.12 mmol/L)

Limit of Detection = 0.29 g/dL (0.18 mmol/L)

This corresponds to a Limit of Blank of 15 mmol/mol (IFCC) and 3.5 % HbA1c (DCCT/NGSP) and a Limit of Detection of 22 mmol/mol (IFCC) and 4.2 % HbA1c (DCCT/NGSP) at a typical hemoglobin concentration of 13.2 g/dL (8.2 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 (acc. to IFCC): 20-42 mmol/mol HbA1c[30,31,32,33]

Protocol 2 (% HbA1c acc. to DCCT/NGSP): 4.0-6.0 % HbA1c

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.[29,34] 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.[29,34]

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.[23,35]

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 CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The experiment was performed with 3 different reagent lots and on 3 different **cobas c** 503 instruments. Data have been evaluated using variance component analysis including sources of variation instrument (between instrument), lot (between lot), day (between day), and run (between run), nested in this sequence and run being the source of variation above error (repeatability). The following results were obtained (data based on DCCT/NGSP values):

Whole blood application

cobas c 503 analyzer 1

	Repeatability	*Intermediate precision*			
		Between run	*Between day*	*Between lot*	*Total*
Sample Mean	***SD % CV***	***SD % CV***	***SD % CV***	***SD % CV***	***SD % CV***
Human 1 4.87 % HbA1c	0.038 0.8	0.000 0.0	0.043 0.9	0.045 0.9	0.073 1.5
Human 2 6.60 % HbA1c	0.026 0.4	0.015 0.2	0.030 0.4	0.071 1.1	0.083 1.3
Human 3 7.37 % HbA1c	0.032 0.4	0.018 0.2	0.032 0.4	0.084 1.1	0.097 1.3
Human 4 8.24 % HbA1c	0.039 0.5	0.008 0.1	0.048 0.6	0.089 1.1	0.108 1.3
Human 5 12.59 % HbA1c	0.056 0.4	0.025 0.2	0.061 0.5	0.154 1.2	0.177 1.4
Human 6 14.69 % HbA1c	0.079 0.5	0.043 0.3	0.067 0.5	0.168 1.1	0.202 1.4
Human 7 12.34 % HbA1c	0.062 0.5	0.032 0.3	0.057 0.5	0.152 1.2	0.176 1.4
Human 8 13.14 % HbA1c	0.061 0.5	0.015 0.1	0.055 0.4	0.165 1.3	0.185 1.4
PC HbA1c norm 5.51 % HbA1c	0.029 0.5	0.002 0.0	0.039 0.7	0.044 0.8	0.066 1.2
PC HbA1c path 11.20 % HbA1c	0.055 0.5	0.011 0.1	0.057 0.5	0.129 1.2	0.152 1.4

cobas c 503 analyzer 2

	Repeatability	*Intermediate precision*			
		Between run	*Between day*	*Between lot*	*Total*
Sample Mean	***SD % CV***	***SD % CV***	***SD % CV***	***SD % CV***	***SD % CV***
Human 1 4.88 % HbA1c	0.032 0.7	0.013 0.3	0.026 0.5	0.031 0.6	0.053 1.1
Human 2 6.58 % HbA1c	0.031 0.5	0.010 0.2	0.025 0.4	0.077 1.2	0.087 1.3

	Repeatability	Intermediate precision			
		Between run	Between day	Between lot	Total
Sample Mean	SD % CV	SD % CV	SD % CV	SD % CV	SD % CV
Human 3 7.35 % HbA1c	0.040 0.5	0.000 0.0	0.029 0.4	0.091 1.2	0.103 1.4
Human 4 8.21 % HbA1c	0.042 0.5	0.011 0.1	0.035 0.4	0.106 1.3	0.120 1.5
Human 5 12.53 % HbA1c	0.070 0.6	0.029 0.2	0.047 0.4	0.164 1.3	0.186 1.5
Human 6 14.62 % HbA1c	0.095 0.6	0.035 0.2	0.091 0.6	0.198 1.4	0.240 1.6
Human 7 12.25 % HbA1c	0.078 0.6	0.000 0.0	0.035 0.3	0.176 1.4	0.195 1.6
Human 8 13.06 % HbA1c	0.073 0.6	0.009 0.1	0.052 0.4	0.163 1.2	0.186 1.4
PC HbA1c norm 5.51 % HbA1c	0.036 0.6	0.000 0.0	0.028 0.5	0.044 0.8	0.063 1.1
PC HbA1c path 11.13 % HbA1c	0.062 0.6	0.033 0.3	0.043 0.4	0.162 1.5	0.182 1.6

cobas c 503 analyzer 3

	Repeatability	Intermediate precision			
		Between run	Between day	Between lot	Total
Sample Mean	SD % CV	SD % CV	SD % CV	SD % CV	SD % CV
Human 1 4.86 % HbA1c	0.029 0.6	0.000 0.0	0.035 0.7	0.044 0.9	0.064 1.3
Human 2 6.55 % HbA1c	0.031 0.5	0.009 0.1	0.025 0.4	0.080 1.2	0.090 1.4
Human 3 7.31 % HbA1c	0.039 0.5	0.006 0.1	0.026 0.4	0.090 1.2	0.101 1.4
Human 4 8.17 % HbA1c	0.043 0.5	0.012 0.2	0.036 0.4	0.096 1.2	0.111 1.4
Human 5 12.51 % HbA1c	0.079 0.6	0.000 0.0	0.064 0.5	0.154 1.2	0.184 1.5
Human 6 14.62 % HbA1c	0.082 0.6	0.000 0.0	0.107 0.7	0.191 1.3	0.234 1.6
Human 7 12.23 % HbA1c	0.068 0.6	0.028 0.2	0.056 0.5	0.157 1.3	0.182 1.5
Human 8 13.05 % HbA1c	0.083 0.6	0.000 0.0	0.064 0.5	0.162 1.2	0.193 1.5
PC HbA1c norm 5.50 % HbA1c	0.030 0.6	0.006 0.1	0.028 0.5	0.051 0.9	0.066 1.2
PC HbA1c path 11.10 % HbA1c	0.071 0.6	0.000 0.0	0.043 0.4	0.137 1.2	0.160 1.4

Reproducibility - **cobas c** 503 analyzer

	Repeatability	Intermediate precision	
		Between run	Between day
Sample Mean	SD % CV	SD % CV	SD % CV
Human 1 4.87 % HbA1c	0.034 0.7	0.004 0.1	0.036 0.7
Human 2 6.57 % HbA1c	0.029 0.4	0.012 0.2	0.028 0.4
Human 3 7.34 % HbA1c	0.037 0.5	0.009 0.1	0.032 0.4
Human 4 8.20 % HbA1c	0.041 0.5	0.011 0.1	0.042 0.5
Human 5 12.54 % HbA1c	0.069 0.5	0.020 0.2	0.062 0.5
Human 6 14.64 % HbA1c	0.086 0.6	0.028 0.2	0.096 0.7
Human 7 12.27 % HbA1c	0.070 0.6	0.023 0.2	0.055 0.5
Human 8 13.08 % HbA1c	0.073 0.6	0.000 0.0	0.061 0.5
PC HbA1c norm 5.51 % HbA1c	0.032 0.6	0.002 0.0	0.032 0.6
PC HbA1c path 11.14 % HbA1c	0.063 0.6	0.019 0.2	0.054 0.5

Reproducibility - **cobas c** 503 analyzer

	Intermediate precision		
	Between lot	Between instrument	Reproducibility (Total)
Sample Mean	SD % CV	SD % CV	SD % CV
Human 1 4.87 % HbA1c	0.040 0.8	0.007 0.1	0.064 1.3
Human 2 6.57 % HbA1c	0.075 1.1	0.024 0.4	0.090 1.4
Human 3 7.34 % HbA1c	0.087 1.2	0.027 0.4	0.104 1.4
Human 4 8.20 % HbA1c	0.096 1.2	0.031 0.4	0.117 1.4
Human 5 12.54 % HbA1c	0.155 1.2	0.043 0.3	0.187 1.5
Human 6 14.64 % HbA1c	0.181 1.2	0.043 0.3	0.228 1.6
Human 7 12.27 % HbA1c	0.159 1.3	0.055 0.4	0.192 1.6
Human 8 13.08 % HbA1c	0.161 1.2	0.049 0.4	0.194 1.5
PC HbA1c norm 5.51 % HbA1c	0.047 0.8	0.007 0.1	0.065 1.2
PC HbA1c path 11.14 % HbA1c	0.141 1.3	0.047 0.4	0.171 1.5

Hemolysate application

A1CX3

Tina-quant Hemoglobin A1cDx Gen.3

cobas c 503 analyzer 1

	Repeatability	Intermediate precision			
		Between run	Between day	Between lot	Total
Sample Mean	SD % CV	SD % CV	SD % CV	SD % CV	SD % CV
Human 1 4.96 % HbA1c	0.025 0.5	0.006 0.1	0.071 1.4	0.016 0.3	0.077 1.6
Human 2 6.62 % HbA1c	0.027 0.4	0.013 0.2	0.053 0.8	0.059 0.9	0.085 1.3
Human 3 7.32 % HbA1c	0.035 0.5	0.000 0.0	0.053 0.7	0.067 0.9	0.092 1.3
Human 4 8.32 % HbA1c	0.039 0.5	0.009 0.1	0.056 0.7	0.083 1.0	0.108 1.3
Human 5 12.54 % HbA1c	0.057 0.5	0.011 0.1	0.100 0.8	0.203 1.6	0.234 1.9
Human 6 14.77 % HbA1c	0.077 0.5	0.013 0.1	0.148 1.0	0.268 1.8	0.316 2.1
Human 7 12.14 % HbA1c	0.055 0.5	0.023 0.2	0.100 0.8	0.181 1.5	0.215 1.8
Human 8 12.94 % HbA1c	0.072 0.6	0.000 0.0	0.111 0.9	0.188 1.5	0.230 1.8
PC HbA1c norm 5.53 % HbA1c	0.024 0.4	0.009 0.2	0.059 1.1	0.028 0.5	0.071 1.3
PC HbA1c path 10.89 % HbA1c	0.055 0.5	0.026 0.2	0.085 0.8	0.146 1.3	0.179 1.6

cobas c 503 analyzer 2

	Repeatability	Intermediate precision			
		Between run	Between day	Between lot	Total
Sample Mean	SD % CV	SD % CV	SD % CV	SD % CV	SD % CV
Human 1 4.96 % HbA1c	0.027 0.5	0.005 0.1	0.034 0.7	0.015 0.3	0.046 0.9
Human 2 6.59 % HbA1c	0.035 0.5	0.000 0.0	0.038 0.6	0.057 0.9	0.077 1.2
Human 3 7.29 % HbA1c	0.041 0.6	0.000 0.0	0.043 0.6	0.068 0.9	0.090 1.2
Human 4 8.28 % HbA1c	0.039 0.5	0.015 0.2	0.046 0.6	0.093 1.1	0.112 1.4
Human 5 12.43 % HbA1c	0.069 0.6	0.027 0.2	0.038 0.3	0.175 1.4	0.193 1.6
Human 6 14.68 % HbA1c	0.085 0.6	0.011 0.1	0.060 0.4	0.220 1.5	0.243 1.7
Human 7 12.05 % HbA1c	0.063 0.5	0.018 0.1	0.036 0.3	0.163 1.4	0.179 1.5
Human 8 12.85 % HbA1c	0.071 0.6	0.034 0.3	0.053 0.4	0.177 1.4	0.201 1.6
PC HbA1c norm 5.52 % HbA1c	0.030 0.5	0.008 0.1	0.029 0.5	0.024 0.4	0.049 0.9
PC HbA1c path 10.81 % HbA1c	0.074 0.7	0.000 0.0	0.041 0.4	0.134 1.2	0.159 1.5

cobas c 503 analyzer 3

	Repeatability	Intermediate precision			
		Between run	Between day	Between lot	Total
Sample Mean	SD % CV	SD % CV	SD % CV	SD % CV	SD % CV
Human 1 4.94 % HbA1c	0.027 0.5	0.010 0.2	0.031 0.6	0.023 0.5	0.048 1.0
Human 2 6.57 % HbA1c	0.030 0.5	0.004 0.1	0.035 0.5	0.049 0.7	0.067 1.0
Human 3 7.28 % HbA1c	0.032 0.4	0.000 0.0	0.038 0.5	0.054 0.7	0.073 1.0
Human 4 8.28 % HbA1c	0.040 0.5	0.000 0.0	0.041 0.5	0.070 0.8	0.091 1.1
Human 5 12.43 % HbA1c	0.063 0.5	0.024 0.2	0.045 0.4	0.162 1.3	0.181 1.5
Human 6 14.68 % HbA1c	0.075 0.5	0.000 0.0	0.050 0.3	0.240 1.6	0.256 1.7
Human 7 12.07 % HbA1c	0.064 0.5	0.000 0.0	0.039 0.3	0.146 1.2	0.164 1.4
Human 8 12.84 % HbA1c	0.072 0.6	0.000 0.0	0.052 0.4	0.150 1.2	0.174 1.4
PC HbA1c norm 5.49 % HbA1c	0.026 0.5	0.008 0.2	0.035 0.6	0.024 0.4	0.050 0.9
PC HbA1c path 10.78 % HbA1c	0.071 0.7	0.000 0.0	0.048 0.4	0.116 1.1	0.144 1.3

Reproducibility - **cobas c** 503 analyzer

	Repeatability	Intermediate precision	
		Between run	Between day
Sample Mean	SD % CV	SD % CV	SD % CV
Human 1 4.96 % HbA1c	0.026 0.5	0.007 0.1	0.049 1.0
Human 2 6.59 % HbA1c	0.031 0.5	0.006 0.1	0.042 0.6
Human 3 7.30 % HbA1c	0.036 0.5	0.000 0.0	0.045 0.6
Human 4 8.29 % HbA1c	0.039 0.5	0.005 0.1	0.049 0.6
Human 5 12.47 % HbA1c	0.063 0.5	0.022 0.2	0.070 0.6
Human 6 14.71 % HbA1c	0.079 0.5	0.010 0.1	0.098 0.7
Human 7 12.08 % HbA1c	0.061 0.5	0.016 0.1	0.067 0.6
Human 8 12.88 % HbA1c	0.072 0.6	0.017 0.1	0.078 0.6
PC HbA1c norm 5.51 % HbA1c	0.027 0.5	0.008 0.2	0.043 0.8
PC HbA1c path 10.83 % HbA1c	0.067 0.6	0.000 0.0	0.062 0.6

Reproducibility - **cobas c** 503 analyzer

	Intermediate precision		
	Between lot	***Between instrument***	***Reproducibility (Total)***
Sample Mean	***SD % CV***	***SD % CV***	***SD % CV***
Human 1 4.96 % HbA1c	0.019 0.4	0.009 0.2	0.059 1.2
Human 2 6.59 % HbA1c	0.055 0.8	0.023 0.3	0.080 1.2
Human 3 7.30 % HbA1c	0.063 0.9	0.019 0.3	0.088 1.2
Human 4 8.29 % HbA1c	0.082 1.0	0.019 0.2	0.105 1.3
Human 5 12.47 % HbA1c	0.179 1.4	0.061 0.5	0.212 1.7
Human 6 14.71 % HbA1c	0.242 1.6	0.053 0.4	0.278 1.9
Human 7 12.08 % HbA1c	0.163 1.3	0.048 0.4	0.193 1.6
Human 8 12.88 % HbA1c	0.172 1.3	0.055 0.4	0.210 1.6
PC HbA1c norm 5.51 % HbA1c	0.027 0.5	0.022 0.4	0.062 1.1
PC HbA1c path 10.83 % HbA1c	0.132 1.2	0.054 0.5	0.169 1.6

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 15.5 % 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) = 160

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 16.2 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 513 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to values assigned by a secondary reference laboratory of the NGSP (x). Samples were measured individually. Mean difference and 95 % confidence intervals of the differences are shown. Additionally, the linear regression equation and absolute and relative bias calculated at diagnostic important cut points based on the linear regression are given.

Sample size (n) = 171

Mean difference: -0.05 % HbA1c

Lower 95 % confidence interval of differences: -0.41 % HbA1c

Upper 95 % confidence interval of differences: 0.32 % HbA1c

Passing-Bablok[36]: y = 1.030x - 0.271; r = 0.998

Bias at 5 % HbA1c: -0.121 % HbA1c (-2.4 % relative bias)

Bias at 6.5 % HbA1c: -0.0760 % HbA1c (-1.2 % relative bias)

Bias at 8 % HbA1c: -0.0310 % HbA1c (-0.4 % relative bias)

The sample concentrations were between 4.8 % and 19.4 % 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 15.3 % 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 15.8 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 513 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to values assigned by a secondary reference laboratory of the NGSP (x). Samples were measured individually. Mean difference and 95 % confidence intervals of the differences are shown. Additionally, the linear regression equation and absolute and relative bias calculated at diagnostic important cut points based on the linear regression are given.

Sample size (n) = 173

Mean difference: 0.05 % HbA1c

Lower 95 % confidence interval of differences: -0.34 % HbA1c

Upper 95 % confidence interval of differences: 0.43 % HbA1c

Passing-Bablok: y = 1.016x - 0.0479; r = 0.998

Bias at 5 % HbA1c: 0.0311 % HbA1c (0.6 % relative bias)

Bias at 6.5 % HbA1c: 0.0547 % HbA1c (0.8 % relative bias)

Bias at 8 % HbA1c: 0.0784 % HbA1c (1.0 % relative bias)

The sample concentrations were between 4.8 % and 19.4 % HbA1c (DCCT/NGSP values).

Hb Variants

Heterozygous presence of the most common hemoglobin variants (HbAS, HbAC, HbAD, HbAE) does not interfere. Significant interference was defined as ≥ 7 % change in HbA1c value in the presence of the hemoglobin variant relative to control.

Variant Type	Number of Samples	% Variant	HbA1c %
HbS	30	35 - 41 % S	4.35 - 12.7
HbC	30	28 - 37 % C	4.90 - 14.1
HbE	30	24 - 27 % E	5.17 - 10.0
HbD	29	36 - 42 % D	5.17 - 9.70
HbA2	15	4.3 - 6.5 % A2	5.10 - 9.80
Elevated HbF	19	3.2 - 39 % F	6.10 - 9.30

Percent Relative Bias from Reference Method at Low and High Concentrations of HbA1c Samples				
	Around 6.5 % HbA1c		Around 9 % HbA1c	
Hb Variant	Relative % Difference	Range	Relative % Difference	Range
HbS	-2.5	-7.2 - 3.2	-4.0	-9.3 - (-2.0)
HbC	-3.9	-7.7 - 2.8	-6.0	-4.6 - (-3.6)
HbE	-0.1	-5.5 - 5.7	-1.2	-5.2 - 0.6
HbD	-1.8	-4.5 - 3.0	-2.6	-3.3 - 0.2
HbA2	-1.0	-4.1 - 2.7	0.4	-2.2 - 1.1
HbF	Specimens containing high amounts of HbF (> 7 %) may yield lower than expected HbA1c values.			

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.[37] 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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Symbols

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

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

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