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A1C-3

cobas®

Tina-quant Hemoglobin A1c Gen.3 - Hemolysate and Whole Blood Application

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05336163 190	Tina-quant Hemoglobin A1c Gen.3 (150 tests)	System ID 07 7455 3	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

04528417 190	Calibrator f.a.s. HbA1c (3 x 2 mL)	Code 674	
05479207 190	PreciControl HbA1c norm (4 × 1 mL)	Code 208	
05912504 190	PreciControl HbA1c path (4 × 1 mL)	Code 209	
04528182 190	Hemolyzing Reagent Gen.2 (51 mL)*	System ID 07 6873 1	
11488457 122	HbA1c Hemolyzing Reagent for Tina-quant HbA1c (1000 mL)	For Hemolysate Application only	

* The value encoded in the instrument settings is 45 mL to account for the dead volume of the bottles.

English

System information

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

HB-W3:	ACN 871	Hemoglobin (Hb)
A1-W3:	ACN 881	Hemoglobin A1c (HbA1c)
RWD3:	ACN 891	Ratio % HbA1c (acc. to DCCT/NGSP)
A1CD2:	ACN 952	Hemolyzing reagent

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

HB-H3:	ACN 841	Hemoglobin (Hb)
A1–H3:	ACN 851	Hemoglobin A1c (HbA1c)
RHD3:	ACN 861	Ratio % HbA1c (acc. to DCCT/NGSP)
A1CD2:	ACN 952	Hemolyzing reagent

Intended use

In vitro test for the quantitative determination of mmol/mol hemoglobin (IFCC) and % hemoglobin A1c (DCCT/NGSP) in whole blood or hemolysate on Roche/Hitachi **cobas c** systems. HbA1c determinations are useful 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[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 approximate relationship between HbA1c and mean blood glucose values during the preceding 2 to 3 months was analyzed in several studies. A recent study obtained the following correlation:

IFCC standardization (recalculated acc. to ref. 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

Standardization acc. to DCCT/NGSP[7]

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

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

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.[3,4]

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.[6]

Test principle[9,10,11]

This method uses TTAB[a)] 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, HbAD, HbAE) can be determined using this assay.[12,13,14]

a) TTAB = 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; detergent; stabilizers; preservatives

R3 Polyhapten Reagent

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

R1 is in position A and R3 is in position C. Position B contains H_2O for technical reasons.

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 dust/fume/gas/mist/vapours/spray.

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

A1C-3

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

Hemolyzing reagent

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

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.

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

1. Allow blood specimen and Hemolyzing Reagent for Tina-quant HbA1c to equilibrate at room temperature before use.

2. Moderately mix the sample immediately prior to pipetting to ensure a homogeneous mixture of erythrocytes. Take care to avoid the formation of foam.

3. Dilute the sample with Hemolyzing Reagent for Tina-quant HbA1c (Cat. No. 11488457 122) in the ratio 1:101 (1+100) using one of the following pipetting schemes.

Pipette into tubes:

HbA1c Hemolyzing Reagent for Tina-quant HbA1c	500 µL	1000 µL	2000 µL
Specimen (patient or control)	5 µL	10 µL	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 (approx. 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

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 (HB-W3) and HbA1c (A1-W3)

cobas c 311 **test definition Hb (HB-W3)**

Assay type	1-Point
Reaction time / Assay points	10 / 23

Wavelength (sub/main)	660 / 376 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	2 µL	180 µL
Decreased	5 µL	2 µL	180 µL
Increased	5 µL	2 µL	180 µL

cobas c 311 **test definition HbA1c (A1-W3)**

Assay type	2-Point End		
Reaction time / Assay points	10 / 23-57		
Wavelength (sub/main)	660 / 340 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	2 µL	180 µL
Decreased	5 µL	2 µL	180 µL
Increased	5 µL	2 µL	180 µL

cobas c 501/502 **test definition Hb (HB-W3)**

Assay type	1-Point		
Reaction time / Assay points	10 / 34		
Wavelength (sub/main)	660 / 376 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	2 µL	180 µL
Decreased	5 µL	2 µL	180 µL
Increased	5 µL	2 µL	180 µL

cobas c 501/502 **test definition HbA1c (A1-W3)**

Assay type	2-Point End		
Reaction time / Assay points	10 / 34-70		
Wavelength (sub/main)	660 / 340 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	2 µL	180 µL
Decreased	5 µL	2 µL	180 µL
Increased	5 µL	2 µL	180 µL

Ratio definition for mmol/mol HbA1c and % HbA1c calculation
Protocol 1 (mmol/mol HbA1c acc. to IFCC):

Abbreviated ratio name	**RWI3**
Equation	(A1-W3/HB-W3) × 1000
Unit	mmol/mol

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

Abbreviated ratio name	**RWD3 (891)**
Equation	(A1-W3/HB-W3) × 91.5 + 2.15
Unit	%

Protocol 2 is already implemented in the application (ACN 891). The mmol/mol HbA1c values according to Protocol 1 (IFCC) must be manually calculated according to the above equation. If requested a calculated test with the formula in protocol 1 can be programmed under Utility > calculated test on the **cobas c** 311 analyzer and on the **cobas c** 501/502 analyzers. Please use the following settings:

Sample Type	Supernt.
Unit of Measure	mM/M
Report Name	HbA1c Gen.3 IFCC
Item	RWI3
Formula	(A1-W3/HB-W3) x 1000

The ratio for HbA1c (mmol/mol HbA1c acc. to IFCC and % HbA1c acc. to DCCT/NGSP) will be automatically calculated after result output of both tests. It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places, which can be entered in the editable field “expected values”.

Hemolysate Application for Hb (HB-H3) and HbA1c (A1-H3)

cobas c 311 **test definition Hb (HB-H3)**

Assay type	1-Point		
Reaction time / Assay points	10 / 23		
Wavelength (sub/main)	660 / 376 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*

Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 311 **test definition HbA1c (A1-H3)**

Assay type	2-Point End		
Reaction time / Assay points	10 / 23-57		
Wavelength (sub/main)	660 / 340 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 501/502 **test definition Hb (HB-H3)**

Assay type	1-Point		
Reaction time / Assay points	10 / 34		
Wavelength (sub/main)	660 / 376 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 501/502 **test definition HbA1c (A1-H3)**

Assay type	2-Point End		
Reaction time / Assay points	10 / 34-70		
Wavelength (sub/main)	660 / 340 nm		
Reaction direction	Increase		
Unit	mmol/L (g/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	-	
R3	24 µL	-	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

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

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

Abbreviated ratio name	**RHI3**
Equation	(A1-H3/HB-H3) x 1000
Unit	mmol/mol

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

Abbreviated ratio name	**RHD3 (861)**
Equation	(A1-H3/HB-H3) x 91.5 + 2.15
Unit	%

Protocol 2 is already implemented in the application (ACN 861). The mmol/mol HbA1c values according to Protocol 1 (IFCC) must be manually calculated according to the above equation. If requested a calculated test with the formula in protocol 1 can be programmed under *Utility* > calculated test on the **cobas c** 311 analyzer and on the **cobas c** 501/502 analyzers. Please use the following settings:

Sample Type	Supernt.
Unit of Measure	mM/M
Report Name	HbA1c Gen.3 IFCC
Item	RHI3
Formula	(A1-H3/HB-H3) x 1000

The ratio for HbA1c (mmol/mol HbA1c acc. to IFCC and % HbA1c acc. to DCCT/NGSP) will be automatically calculated after result output of both tests. It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places, which can be entered in the editable field “expected values”.

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	Spline
Calibration frequency	Hb and HbA1c: full calibration is recommended - after 29 days during shelf life - after reagent lot change - as required following quality control procedures Always calibrate both assays (Hb and HbA1c) in parallel. Automatic calibration at QC failure should be deactivated.

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

Traceability: This method has been standardized against the approved IFCC reference method for the measurement of HbA1c in human blood [15,16] and can be transferred to results traceable to DCCT/NGSP by calculation.

Note for Whole Blood and Hemolysate Application

Enter the assigned lot-specific and application-specific value of the calibrator. Use the appropriate C.f.a.s. HbA1c calibrator only.

The **cobas c** Hemolyzing Reagent Gen.2 pack, 51 mL, Cat. No. 04528182 190, 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. 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.

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 for Whole Blood and Hemolysate Application[12,13,17,18,19,20,21,22,23,24]

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.[22] 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 (> 10 %) may result in lower than expected mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP).[13,24]

6. mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) are not suitable for the diagnosis of gestational diabetes.[25]

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.[25]

Criterion: Recovery within ± 10 % of initial value.

Icterus:[21] 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):[21] 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 up to a glucose level 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.[26,27]

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.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases.

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

Limits and ranges

Measuring range

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

HbA1c: 0.186-1.61 mmol/L (0.3-2.6 g/dL)

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 (13.2 g/dL).

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 (0.50 g/dL)

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

HbA1c:

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

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

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A 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[28]

Protocol 2 (% HbA1c acc. to DCCT/NGSP): 4.8-5.9 % HbA1c[28]

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.[25,29] 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.[25,29]

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.[20,19]

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. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained (data based on DCCT/NGSP values):

Whole Blood Application:

Repeatability	Mean	SD	CV
	% HbA1c	%	%
PreciControl HbA1c norm	5.3	0.07	1.3
PreciControl HbA1c path	9.9	0.11	1.1
Human sample 1	4.4	0.07	1.6
Human sample 2	5.6	0.09	1.6
Human sample 3	8.0	0.08	1.0
Human sample 4	10.6	0.11	1.1
Intermediate precision	Mean	SD	CV
	% HbA1c	%	%
PreciControl HbA1c norm	5.3	0.08	1.4
PreciControl HbA1c path	9.9	0.15	1.5
Human sample 1	4.4	0.09	1.9
Human sample 2	5.6	0.11	2.0
Human sample 3	8.0	0.11	1.4
Human sample 4	10.6	0.16	1.5

Hemolysate Application:

Repeatability	Mean	SD	CV
	% HbA1c	%	%
PreciControl HbA1c norm	5.1	0.07	1.3
PreciControl HbA1c path	10.2	0.10	1.0
Human sample 1	4.3	0.06	1.4
Human sample 2	5.6	0.07	1.2
Human sample 3	8.2	0.08	1.0
Human sample 4	10.9	0.11	1.0
Intermediate precision	Mean	SD	CV
	% HbA1c	%	%
PreciControl HbA1c norm	5.1	0.11	2.2
PreciControl HbA1c path	10.2	0.21	2.0
Human sample 1	4.3	0.10	2.3
Human sample 2	5.6	0.09	1.6
Human sample 3	8.2	0.16	1.9
Human sample 4	10.9	0.22	2.0

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

Method comparison

Evaluation of method comparison data is according to 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** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared with those determined using the corresponding reagent with the hemolysate application on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 80

Mean difference	0.07 % HbA1c
Lower 95 % confidence interval of differences	-0.27 % HbA1c
Upper 95 % confidence interval of differences	0.42 % HbA1c

The sample concentrations were between 4.7 % and 9.8 % (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared with those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the whole blood application (x).

Sample size (n) = 82

Mean difference	0.07 % HbA1c
Lower 95 % confidence interval of differences	-0.50 % HbA1c
Upper 95 % confidence interval of differences	0.65 % HbA1c

The sample concentrations were between 5.0 % and 9.9 % (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared with those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the hemolysate application on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 80

Mean difference	-0.09 % HbA1c
Lower 95 % confidence interval of differences	-0.46 % HbA1c
Upper 95 % confidence interval of differences	0.28 % HbA1c

The sample concentrations were between 4.7 % and 9.8 % (DCCT/NGSP values).

Hemolysate Application:

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared with those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the hemolysate application on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 111

Mean difference	-0.19 % HbA1c
Lower 95 % confidence interval of differences	-0.52 % HbA1c
Upper 95 % confidence interval of differences	0.14 % HbA1c

The sample concentrations were between 4.6 % and 9.9 % (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application (y) were compared with those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the whole blood application (x).

Sample size (n) = 84

Mean difference	-0.06 % HbA1c
Lower 95 % confidence interval of differences	-0.53 % HbA1c
Upper 95 % confidence interval of differences	0.41 % HbA1c

The sample concentrations were between 5.5 % and 9.9 % (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application (y) were compared with those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the hemolysate application on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 111

Mean difference	-0.35 % HbA1c
Lower 95 % confidence interval of differences	-0.68 % HbA1c
Upper 95 % confidence interval of differences	-0.02 % HbA1c

The sample concentrations were between 4.7 % and 9.9 % (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application (y) were compared with those determined using the Tina-quant Hemoglobin A1c Gen.2 reagent with the hemolysate application (x).

Sample size (n) = 113

Mean difference	-0.10 % HbA1c
Lower 95 % confidence interval of differences	-0.49 % HbA1c
Upper 95 % confidence interval of differences	0.31 % HbA1c

The sample concentrations were between 4.8 % and 9.7 % (DCCT/NGSP values).

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

Analytical specificity for Whole Blood and Hemolysate Application

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 (> 10 %) 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.[30] 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 (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

CE 0123

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