



05336996001V4.0

# A1C-3

**cobas**<sup>®</sup>

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

### Order information

REF	CONTENT	Analyzer(s) on which kit(s) can be used
05336180190	Tina-quant Hemoglobin A1c Gen.3 (2 x 100 tests)	<b>cobas c 111</b>
Materials required (but not provided):		
04528417190	C.f.a.s. HbA1c (3 x 2 mL)	Code 674
05479207190	PreciControl HbA1c norm (4 x 1 mL)	Code 208
05912504190	PreciControl HbA1c path (4 x 1 mL)	Code 209
05007232190	Hemolyzing Reagent Gen.2 (8 x 6.3 mL)	Code 952
11488457122	Hemolyzing Reagent for Tina-quant HbA1c (1000 mL)	
11930630001	Chimneys	

### English

#### System information

Whole Blood **DCCT (%)**: ACN 891 **A1W3D** and ACN 871 **HBW3D**  
 Hemolysate **DCCT (%)**: ACN 861 **A1H3D** and ACN 841 **HBH3D**  
 Whole Blood **IFCC (mmol/mol)**: ACN 264 **A1W3I** and ACN 265 **HBW3I**  
 Hemolysate **IFCC (mmol/mol)**: ACN 251 **A1H3I** and ACN 231 **HBH3I**

#### Intended use

In vitro test for the quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in whole blood and hemolysates prepared from whole blood on the **cobas c 111** system. 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.<sup>1</sup> Hb consists of a variety of subfractions and derivatives, including glycosylated hemoglobins, formed by the attachment of various sugars to the Hb molecule. The set of glycosylated 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  $\beta$ -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.<sup>2</sup>

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.<sup>3,4</sup> 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.<sup>2</sup>

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

According to IFCC standardization<sup>8</sup>

• 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<sup>9</sup>

• 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.<sup>10,11,12,13,14,15,16,17,18,19</sup>

#### Test principle<sup>20,21,22</sup>

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 glycosylated at the  $\beta$ -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.<sup>23,24,25</sup>

\*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 SR (buffer/polyhapten) and start of reaction:

The polyhapten 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) x 1000



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

$$\text{HbA1c (\%)} = (\text{HbA1c/Hb}) \times 91.5 + 2.15$$

### 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)  $\geq$  0.5 mg/mL; detergents; stabilizers; preservative
- SR** Polyhapten reagent  
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c polyhapten:  $\geq$  8  $\mu\text{g/mL}$ ; detergents; stabilizers; preservative

### 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

Under conditions of extreme humidity, condensation may lead to a dilution of the reagent that affects the measurements. Hence under environmental conditions in which temperature and humidity are equal to, or in excess of 25 °C/80 %, 28 °C/70 %, 30 °C/60 % or 32 °C/55 % a chimney (Cat. No. 11930630 001) should be used to reduce the condensation rate. Place a white chimney in R1 and a black chimney in SR. The chimneys can be reused for reagent bottles within the same kit. However, to avoid contamination of the reagent with detergent or dilution of the reagent with water it is not permitted to wash the chimneys before reuse.

### Storage and stability

- Shelf life at 2-8 °C: See expiration date on reagent
- 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 or capillary blood or hemolysate  
The only acceptable anticoagulants are Li-Heparin, K<sub>2</sub>-EDTA, K<sub>3</sub>-EDTA, Fluoride/Na<sub>2</sub>-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

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.

### Whole blood application

Whole blood samples must be placed on the instrument in uncapped primary tubes and not in (micro) tubes.

### Hemolysate application

Manual hemolysate preparation:

- Allow blood specimen and Hemolyzing Reagent for Tina-quant HbA1c (Cat. No. 11488457122) to equilibrate at room temperature before use.
- Moderately mix the sample immediately prior to pipetting, to ensure homogeneous mixture of erythrocytes. Take care to avoid the formation of foam.
- 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  $\mu\text{L}$**

Specimen (patient or control): **5  $\mu\text{L}$**

or

Hemolyzing Reagent for Tina-quant HbA1c: **1000  $\mu\text{L}$**

Specimen (patient or control): **10  $\mu\text{L}$**

or

Hemolyzing Reagent for Tina-quant HbA1c: **2000  $\mu\text{L}$**

Specimen (patient or control): **20  $\mu\text{L}$**

- Mix using a vibration mixer or by gentle swirling.
- 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

### 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.



**Tina-quant Hemoglobin A1c Gen.3 - Whole blood and Hemolysate Application****Whole Blood applications for Hb and HbA1c****cobas c 111 test definition Hb (HBW3I or HBW3D)**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	378/659 nm
Calc. first/last	6/16
Unit	mmol/L
Reaction mode	R1-S

**Pipetting parameters**

Hb	Diluent (H <sub>2</sub> O)	
R1	120 µL	
Sample	6 µL	0 µL
Total volume	126 µL	

**cobas c 111 test definition HbA1c (A1W3I or A1W3D)**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	16/34
Unit	mmol/L
Reaction mode	R1-S-SR

**Pipetting parameters**

HbA1c	Diluent (H <sub>2</sub> O)	
R1	120 µL	
Sample	6 µL	0 µL
SR	24 µL	0 µL
Total volume	150 µL	

**Hemolysate applications for Hb and HbA1c****cobas c 111 test definition Hb (HBH3I or HBH3D)**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	378/659 nm
Calc. first/last	6/16
Unit	mmol/L
Reaction mode	R1-S

**Pipetting parameters**

Hb	Diluent (H <sub>2</sub> O)	
R1	120 µL	
Sample	6 µL	0 µL
Total volume	126 µL	

**cobas c 111 test definition HbA1c (A1H3I or A1H3D)**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	340/659 nm

Calc. first/last	16/34
Unit	mmol/L
Reaction mode	R1-S-SR

**Pipetting parameters**

HbA1c	Diluent (H <sub>2</sub> O)	
R1	120 µL	
Sample	6 µL	0 µL
SR	24 µL	0 µL
Total volume	150 µL	

**Calibration**

Hb	
Calibrator	C.f.a.s. HbA1c
HbA1c	
Calibrator	C.f.a.s. HbA1c
Calibration dilution ratio	1:1, 1:1.5, 1:2.1, 1:3, 1:6, 1:15, performed automatically by the instrument
Calibrator diluent	Hemolyzing Reagent Gen.2
Calibration frequency	Each lot, every 14 days, and 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 approved IFCC reference method for the measurement of HbA1c in human blood<sup>26,27</sup> and can be transferred to results traceable to DCCT/NGSP by calculation.

**Note**

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 111** Hemolyzing Reagent Gen.2 (Cat. No. 05007232 190) needs to be available on the analyzer. Otherwise the calibration cannot be performed.

**Quality control**

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

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

**Note**

HbA1c controls carry a declaration for HbA1c ratio only. No declarations for Hb and HbA1c concentrations are provided. HbA1c controls must be included in the **cobas c 111** systems Quality Control Program. For the hemolysate test the HbA1c controls must be hemolysed manually before using them for the Quality Control Program. Assigned target values in mmol/mol HbA1c according to IFCC are not included in the control barcodes and must be entered by the customer manually.

**Calculation**

The **cobas c 111** analyzer automatically calculates the ratio between A1c and Hb of each sample.

**Limitations - interference**<sup>23,24,28,29,30,31,32,33,34,35</sup>

- 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.
- The test is designed only for accurate and precise measurement of mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP). The individual results for total Hb and HbA1c concentration should not be reported.



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- 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.<sup>33</sup> 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.
- Any cause of shortened erythrocyte survival 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. 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.
- Glycated HbF is not detected by the assay as it does not contain the glycosylated  $\beta$ -chain that characterizes HbA1c. However, HbF is measured in the total Hb assay and as a consequence, specimens containing high amounts of HbF ( $\geq 10\%$ ) may result in lower than expected mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP).<sup>24,35</sup>
- mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) are not suitable for diagnosis of gestational diabetes.<sup>36</sup>
- In very rare cases of rapidly evolving type 1 diabetes the increase of 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.<sup>36</sup>

Criterion: Recovery within  $\pm 10\%$  of initial value.

Icterus: No significant interference up to a conjugated and unconjugated bilirubin concentration of 1026  $\mu\text{mol/L}$  or 60 mg/dL.

Lipemia: No significant interference up to an Intralipid concentration of 600 mg/dL. There is poor correlation between the 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 up to a rheumatoid factor level of 750 IU/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>37,38</sup>

Other: No cross reactions with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin, glycosylated 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 the **cobas c 111** analyzer. For information about test combinations requiring special wash steps, please refer to the latest version of the carry-over evasion list found with the CLEAN Method Sheet and the operator's manual for further instructions.

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

### Limits and ranges

#### Measuring range

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

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

\*The measuring range for HbA1c lies between 0.186 mmol/L and the concentration of the highest standard. The test range stated above is based on a typical calibrator value of 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 (13.2 g/dL).

In rare cases of ">test rng" flags that might occur with the whole blood application remix the whole blood sample and repeat the analysis with the same settings.

### Lower limits of measurement

Limit of Blank and Limit of Detection

Hb:	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 95<sup>th</sup> percentile value from  $n \geq 60$  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 %).

### Expected values

**Protocol 1 (mmol/mol HbA1c acc. to IFCC):** 29-42 mmol/mol HbA1c<sup>39</sup>

**Protocol 2 (% HbA1c acc. to DCCT/NGSP):** 4.8-5.9 % HbA1c<sup>39</sup>

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.<sup>36,40</sup> 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 a risk of developing diabetes.<sup>36,40</sup>

HbA1c levels may reach 195 mmol/mol HbA1c (IFCC) or 20 % HbA1c (DCCT/NGSP) and more 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 HbA1c (IFCC) or 7 % HbA1c (DCCT/NGSP) meet the goal of the American Diabetes Association.<sup>30,31</sup>

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 **cobas c 111** analyzer are given below. Results obtained in individual laboratories may differ.

### Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained on the **cobas c 111** analyzer (data based on DCCT/NGSP values):

### Whole Blood application

Repeatability	Mean % HbA1c	SD % HbA1c	CV %
PreciControl HbA1c norm	5.63	0.04	0.7
PreciControl HbA1c path	10.7	0.06	0.6
Human sample 1	5.21	0.04	0.7
Human sample 2	6.22	0.04	0.7
Human sample 3	7.34	0.05	0.7



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<i>Repeatability</i>	<i>Mean % HbA1c</i>	<i>SD % HbA1c</i>	<i>CV %</i>
Human sample 4	8.44	0.05	0.6
Human sample 5	11.0	0.06	0.5

<i>Intermediate precision</i>	<i>Mean % HbA1c</i>	<i>SD % HbA1c</i>	<i>CV %</i>
PreciControl HbA1c norm	5.63	0.08	1.5
PreciControl HbA1c path	10.7	0.10	0.9
Human sample 1	5.21	0.07	1.4
Human sample 2	6.22	0.07	1.1
Human sample 3	7.34	0.09	1.2
Human sample 4	8.44	0.09	1.1
Human sample 5	11.0	0.10	0.9

**Hemolysate application**

<i>Repeatability</i>	<i>Mean % HbA1c</i>	<i>SD % HbA1c</i>	<i>CV %</i>
PreciControl HbA1c norm	5.76	0.06	1.0
PreciControl HbA1c path	10.5	0.06	0.6
Human sample 1	4.99	0.07	1.5
Human sample 2	6.42	0.08	1.3
Human sample 3	10.2	0.07	0.7
Human sample 4	7.44	0.07	0.9
Human sample 5	7.69	0.05	0.7

<i>Intermediate precision</i>	<i>Mean % HbA1c</i>	<i>SD % HbA1c</i>	<i>CV %</i>
PreciControl HbA1c norm	5.76	0.12	2.1
PreciControl HbA1c path	10.5	0.19	1.8
Human sample 1	4.99	0.11	2.2
Human sample 2	6.42	0.16	2.5
Human sample 3	10.2	0.18	1.8
Human sample 4	7.44	0.14	1.9
Human sample 5	7.69	0.13	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** 111 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared to those determined using the same reagent with the whole blood application on a COBAS INTEGRA 400 plus analyzer (x).

Sample size (n) = 57

Mean difference: 0.22 % HbA1c

Lower 95 % confidence interval of differences: -0.22 % HbA1c

Upper 95 % confidence interval of differences: 0.66 % HbA1c

The sample concentrations were between 4.86 % and 12.2 % HbA1c (DCCT/NGSP values).

**Hemolysate application:**

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 111 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application (y) were compared to those determined using the same reagent with the hemolysate application on a COBAS INTEGRA 400 plus analyzer (x).

Sample size (n) = 57

Mean difference: 0.19 % HbA1c

Lower 95 % confidence interval of differences: -0.16 % HbA1c

Upper 95 % confidence interval of differences: 0.54 % HbA1c

The sample concentrations were between 4.58 % and 11.8 % HbA1c (DCCT/NGSP values).

**Analytical specificity**

Hb derivatives Labile HbA1c (pre-HbA1c), acetylated Hb, and carbamylated Hb do not affect the assay result.

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 the International Diabetes Federation (IDF) HbA1c results should be reported in parallel, both in mmol/mol HbA1c (IFCC) and % HbA1c (DCCT/NGSP) values.<sup>41</sup> 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.

To ensure parallel reporting of results in both, IFCC units (mmol/mol) and DCCT/NGSP units (%) on the **cobas c** 111 analyzer without having to request the assay twice, an additional ratio must be defined on the analyzer. Please contact your local Roche Diagnostics support if you require any assistance with programming this ratio; separate customer information is available on request.

Analyzer software version 3.0 and later is a prerequisite for reporting the new IFCC units (mmol/mol).

**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.

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:

	Contents of kit
	Reagent
	Volume for reconstitution
	Global Trade Item Number

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

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Additions, deletions or changes are indicated by a change bar in the margin.

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# A1C-3

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