A1C-3

cobas®

Tina-quant Hemoglobin A1c Gen.3 - Whole blood application - Standardized according to IFCC transferable to DCCT/NGSP

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 INTEGRA 400 plus
04528417 190	C.f.a.s. HbA1c (3 × 2 mL)	System-ID 07 6852 9	
05479207 190	PreciControl HbA1c norm (4 × 1 mL)	System-ID 07 7477 4	
05912504 190	PreciControl HbA1c path (4 × 1 mL)	System-ID 07 7478 2	
04528328 190	COBAS INTEGRA Hemolyzing Reagent Gen.2 (6 × 10 mL)	System-ID 07 6851 0	

English

System information

Multitest A1CW3, test ID 0-264

Test HB-W3, test ID 0-265; test A1-W3, test ID 0-266

Ratio RWD3, test ID 0-275 (% HbA1c acc. to DCCT/NGSP)

Ratio RWI3, test ID 0-274 (mmol/mol HbA1c acc. to IFCC)

Profile PA1W3, test ID 0-273

Intended use

In vitro test for the quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in whole blood on Roche clinical chemistry analyzers. 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[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 to transport 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 nonenzymatic 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 × HbA1c (mmol/mol) + 0.834

or

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

Standardization acc. to DCCT/NGSP[1]

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

or

• Estimated average glucose [mg/dL] = 28.7 × 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 of 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]

The anticoagulated whole blood specimen is hemolyzed automatically on the COBAS INTEGRA 400 plus analyzer with COBAS INTEGRA Hemolyzing Reagent Gen.2. 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 diabetic patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, HbAE) can be determined by this assay.[12,13]

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, complex formation does not take place.
- Addition of SR (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

a) TTAB = Tetradecyltrimethylammonium bromide

Reagents - working solutions

R1 Antibody reagent
MES[b)] buffer: 0.025 mol/L; TRIS[c)] buffer: 0.015 mol/L, pH 6.2; HbA1c antibody (ovine serum): ≥ 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: ≥ 8 µg/mL; detergents; stabilizers; preservative

b) MES = 2-morpholinoethane sulfonic acid

c) TRIS = Tris(hydroxymethyl)-aminomethane

R1 is in position A and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

A1C-3

cobas®

Tina-quant Hemoglobin A1c Gen.3 - Whole blood application - Standardized according to IFCC transferable to DCCT/NGSP

Storage and stability

Reagent

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	4 weeks

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

Hemolyzing reagent

Shelf life at 2-8 °C	See expiration date on bottle label
COBAS INTEGRA 400 plus system	
On-board in use, ISE rack, closed bottles	4 weeks
On-board in use, multi rack, open bottles	2 days

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

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.

Stability:[14]	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.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

COBAS INTEGRA Hemolyzing Reagent Gen.2, Cat. No. 04528328190, system-ID 07 6851 0

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.

COBAS INTEGRA 400 plus test definition Hb

Abbreviated test name	HB-W3
Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S
Reaction direction	Increase
Wavelength A/B	378/659 nm
Calc. first/last	17/33
Predilution factor	100
Unit	mmol/L

Pipetting parameters

Hb		Diluent (H_2O)
R1	120 µL	
Sample	6 µL	0 µL
Total volume	126 µL	

COBAS INTEGRA 400 plus test definition HbA1c

Abbreviated test name	A1-W3
Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	33/57
Predilution factor	100
Unit	mmol/L

Pipetting parameters

HbA1c		Diluent (H_2O)
R1	120 µL	
Sample	6 µL	0 µL
SR	24 µL	0 µL
Total volume	150 µL	

Ratio definition for mmol/mol HbA1c and % HbA1c calculation

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

Abbreviated ratio name	**RWI3 (0-274)**
Equation	(A1-W3/HB-W3) × 1000
Unit	mM/M

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

Abbreviated ratio name	**RWD3 (0-275)**
Equation	(A1-W3/HB-W3) × 91.5 + 2.15
Unit	%

Use the predefined profile (PA1W3, 0-273) for simultaneous order entry of Hb (HB-W3) and HbA1c (A1-W3) tests from the same sample.

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.

For dual reporting of both mmol/mol HbA1c (IFCC) units as well as % HbA1c (DCCT/NGSP) units please ensure that both ratio tests 0-274 (acc. to IFCC) and 0-275 (acc. to DCCT/NGSP) are activated.

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	COBAS INTEGRA Hemolyzing Reagent Gen.2, Cat. No. 04528328190
Calibration mode	Spline
Calibration replicate	Duplicate recommended
Calibration interval	Each lot, every 29 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[15,16] 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.
COBAS INTEGRA Hemolyzing Reagent Gen.2, 6 x 11 mL,
Cat. No. 04528328190, system-ID 07 6851 0, needs to be available on the analyzer. Otherwise the calibration cannot be carried out.

Quality control

Quality control	PreciControl HbA1c norm
	PreciControl HbA1c path
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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

Pretreat controls in the same way as samples.
HbA1c controls carry a declaration for mmol/mol HbA1c (IFCC) and % HbA1c (DCCT/NGSP) only. No declarations for Hb and HbA1c concentrations are provided. As a consequence, HbA1c controls are handled like samples and cannot be included in the COBAS INTEGRA systems Quality Control Program.

Calculation

Hb
COBAS INTEGRA systems automatically calculate the Hb concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus analyzer).

HbA1c
COBAS INTEGRA systems automatically calculate the HbA1c concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus analyzer).

HbA1c ratio calculation
For calculation of the mmol/mol HbA1c value (IFCC) and the % 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[12,13,17,18,19,20,21,22,23,24]

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 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 diagnosis of gestational diabetes.[25]
7. 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.[25]

Criterion: Recovery within ± 10 % 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): No significant interference up to an Intralipid concentrationof 600 mg/dL. There is poor correlation between the triglycerides concentration and turbidity.

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

Rheumatoid factors: No significant interference up to a rheumatoid factor 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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
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)

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).

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 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

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

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 474 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 a 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 HbA1c (IFCC) or 7 % HbA1c (DCCT/NGSP) meet the goal of the American Diabetes Association.[19,20]

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 INTEGRA analyzers 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 (data based on DCCT/NGSP values):

Repeatability	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
PreciControl HbA1c norm	5.81	0.09	1.6
PreciControl HbA1c path	10.6	0.09	0.8
Human sample 1	5.37	0.09	1.7
Human sample 2	6.43	0.08	1.3
Human sample 3	7.50	0.10	1.4
Human sample 4	8.52	0.08	0.9
Human sample 5	10.9	0.09	0.8

Intermediate precision	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
PreciControl HbA1c norm	5.74	0.12	2.1
PreciControl HbA1c path	10.6	0.18	1.7
Human sample 1	5.35	0.12	2.3
Human sample 2	6.33	0.12	1.9
Human sample 3	7.50	0.14	1.8
Human sample 4	8.52	0.12	1.4
Human sample 5	10.7	0.21	1.9

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.

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a COBAS INTEGRA 400 plus analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application (y) were compared with those determined using the same reagent with the whole blood application on a **cobas c** 501 analyzer (x).

Sample size (n) = 96

Mean difference: 0.03 % HbA1c

Lower 95 % confidence interval of differences: –0.45 % HbA1c

Upper 95 % confidence interval of differences: 0.51 % HbA1c

The sample concentrations were between 4.30 and 11.7 % HbA1c (DCCT/NGSP).

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.[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.

Symbols

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

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

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche