A1C-2

Tina-quant ® Hemoglobin A1c Gen.2

cobas®

• Indicates **cobas c** systems on which reagents can be used

Order information

			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
Tina-quant ® Hemoglobin A1c Gen.2				
150 tests	Cat. No. **04528123** 190	System-ID 07 6850 2	•	•
C.f.a.s. HbA1c (3 x 2 mL)	Cat. No. **04528417** 190	Code 674		
HbA1c Control N (4 x 0.5 mL)	Cat. No. **20764833** 322	Code 357		
HbA1c Control P (4 x 0.5 mL)	Cat. No. **20764841** 322	Code 358		
Hemolyzing Reagent Gen.2 (2 x 22.5 mL)	Cat. No. **04528182** 190	System-ID 07 6873 1		
HbA1c Hemolyzing Reagent for Tina-quant ® HbA1c (1000 mL)	Cat. No. **11488457** 122	For Hemolysate Application only		

English

System information

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

HB-W2:	ACN 870	Hemoglobin (Hb)
A1-W2:	ACN 880	Hemoglobin A1c (HbA1c)
RWI2:	ACN 890	% Ratio
A1CD2:	ACN 952	Hemolyzing reagent

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

HB-H2	ACN 840	Hemoglobin (Hb)
A1–H2	ACN 850	Hemoglobin A1c (HbA1c)
RHI2	ACN 860	% Ratio
A1CD2	ACN 952	Hemolyzing reagent

Intended use

In vitro test for the quantitative determination of percent hemoglobin A1c [HbA1c (%)] in whole blood or in hemolysate on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6,7,8,9]

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)

- Mean plasma glucose [mmol/L] = 1.73 x HbA1c (%) +0.20 or
- Mean plasma glucose [mg/dL] = 31.2 x HbA1c (%) + 3.51

Standardization acc. to DCCT/NGSP[8]

- Mean plasma glucose [mmol/L] = 1.98 × HbA1c (%) - 4.29 or
- Mean plasma glucose [mg/dL] = 35.6 × HbA1c (%) - 77.3

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.[3,5]

For routine clinical use, 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[10,11,12]

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 measured by this assay. Consequently, the metabolic state of patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, HbAE) can be determined using this assay.[13,14]

*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, complex formation does not take place.
- Addition of R2 (buffer/polyhapten) and start of reaction:
  The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be measured 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 percent HbA1c and is calculated from the HbA1c/Hb ratio as follows:

Protocol 1 (acc. to IFCC):
HbA1c (%) = (HbA1c/Hb) × 100

Protocol 2 (acc. to DCCT/NGSP):
HbA1c (%) = (HbA1c/Hb) × 87.6 + 2.27

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; stabilizers; preservatives (liquid)

R2 Polyhapten reagent
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c polyhapten: ≥ 8 µg/mL; stabilizers; preservatives (liquid)

Precautions and warnings

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

Reagent handling

Ready for use.

Storage and stability

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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

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Hemolyzing reagent

Shelf life at 2-8°C: See expiration date on 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.

Venous or capillary blood with anticoagulant.

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

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

Freeze only once. Mix specimen thoroughly before use.

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) 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:[15] 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.
Distilled water
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 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-W2) and HbA1c (A1-W2)

cobas c 311 **test definition Hb (HB-W2)**

Assay type	1 Point
Reaction time / Assay points	10 / 23
Wavelength (sub/main)	660/376 nm
Reaction direction	Increase
Unit	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-W2)**

Assay type	2 Point End
Reaction time / Assay points	10 / 23–57
Wavelength (sub/main)	660/340 nm
Reaction direction	Increase
Unit	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 **test definition Hb (HB-W2)**

Assay type	1 Point
Reaction time / Assay points	10 / 34
Wavelength (sub/main)	660/376 nm
Reaction direction	Increase
Unit	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

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cobas c 501 **test definition HbA1c (A1-W2)**

Assay type	2 Point End	
Reaction time / Assay points	10 / 34-70	
Wavelength (sub/main)	660/340 nm	
Reaction direction	Increase	
Unit	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

Important: To improve the fit of the nonlinear HbA1c calibration curve, a constant and lot independent offset of 0.6 g/dL was added to all calibrator values. This offset is already included in the assigned HbA1c calibrator target values for **cobas c** analyzers and needs to be subtracted from the analyzer's results. Enter the instrument factor of the absolute HbA1c (A1-W2) assay on Roche **cobas c** analyzers as follows:
Calibration => Status Screen => Instrument Factor => Instrument Factor Window => HbA1c (A1-W2) => a = 1.0; b = – 0.6 => update => okay

Ratio definition for HbA1c (%) calculation

Protocol 1 (acc. to IFCC):

Abbreviated ratio name	**RWI2 (890)**
Equation	(A1-W2/HB-W2) × 100
Unit	%

Protocol 2 (acc. to DCCT/NGSP):

Abbreviated ratio name	**RWD2**
Equation	(A1-W2/HB-W2) × 87.6 + 2.27
Unit	%

Protocol 1 is already implemented in the application (ACN 890). Percent HbA1c according to Protocol 2 (DCCT/NGSP) must be manually calculated according to the above equation. If requested the formula (ACN 890) can be modified by using the Administrator Level/EDIT Button.
The ratio for HbA1c (%) will be automatically calculated after result output of both tests.
It is recommended to report HbA1c values to one decimal place, which can be entered in the editable field "expected values".

Hemolysate Application for Hb (HB-H2) and HbA1c (A1–H2)

cobas c 311 **test definition Hb (HB-H2)**

Assay type	1 Point	
Reaction time/Assay points:	10 / 23	
Wavelength (sub/main)	660/376 nm	
Reaction direction	Increase	
Unit	g/dL	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	-
R3	24 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 311 **test definition HbA1c (A1-H2)**

Assay type	2 Point End	
Reaction time/Assay points:	10 / 23–57	
Wavelength (sub/main)	660/340 nm	
Reaction direction	Increase	
Unit	g/dL	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	-
R3	24 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 501 **test definition Hb (HB-H2)**

Assay type	1 Point	
Reaction time/Assay points:	10 / 34	
Wavelength (sub/main)	660/376 nm	
Reaction direction	Increase	
Unit	g/dL	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	-
R3	24 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

cobas c 501 **test definition HbA1c (A1-H2)**

Assay type	2 Point End	
Reaction time/Assay points:	10 / 34 -70	
Wavelength (sub/main)	660/340 nm	
Reaction direction	Increase	
Unit	g/dL	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	-
R3	24 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	-	-
Decreased	5 µL	-	-
Increased	5 µL	-	-

Important: To improve the fit of the nonlinear HbA1c calibration curve a constant and lot independent offset of 0.6 g/dL was added to all calibrator values. This offset is already included in the assigned HbA1c calibrator target values for **cobas c** and finally needs to be subtracted from the analyzer's results. Enter the instrument factor of the absolute HbA1c (A1-H2) assay on Roche **cobas c** analyzers as follows:
Calibration => Status Screen => Instrument Factor => Instrument Factor Window => HbA1c (A1-H2) => a= 1.0; b= – 0.6 => update => okay

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Ratio definition for HbA1c (%) calculation

Protocol 1 (acc. to IFCC):

Abbreviated ratio name	**RHI2 (860)**
Equation	(A1-H2/HB-H2) × 100
Unit	%

Protocol 2 (acc. to DCCT/NGSP):

Abbreviated ratio name	**RHD2**
Equation	(A1-H2/HB-H2) × 87.6 + 2.27
Unit	%

Protocol 1 is already implemented in the application (ACN 860). Percent HbA1c according to Protocol 2 (DCCT/NGSP) must be manually calculated according to the above equation. If requested the formula (ACN 860) can be modified by using the Administrator Level/EDIT Button.
The ratio for HbA1c (%) will be automatically calculated after result output of both tests.
It is recommended to report HbA1c values to one decimal place, 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	RCM
Calibration frequency	Hb and HbA1c: full calibration is recommended - after 29 days during shelf life - after reagent lot change - and as required following quality control procedures Always calibrate both assays (Hb and HbA1c) in parallel. Automatic calibration at QC failure should be deactivated.

Traceability: This method has been standardized against the approved IFCC reference method for the measurement of HbA1c in human blood[16,17] 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, 2 x 22.5 mL, Cat. No. 04528182, 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.
Other suitable control material can be used in addition.
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 limits.

Calculation for Whole Blood and Hemolysate Application
Hb, HbA1c
Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

HbA1c (%)
For calculation of the percent HbA1c value, refer to theTest principle and Ratio definition for HbA1c (%) calculation sections in this method sheet.

Limitations - interference for Whole Blood and Hemolysate Application[13,14,18,19,20,21,22,23,24,25]

1. For diagnostic purposes, HbA1c (%) values 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 HbA1c (%). The individual results for total Hb and HbA1c concentration should not be reported.
3. The test is not intended for the diagnosis of diabetes mellitus or for judging day-to-day glucose control and should not be used to replace daily home testing of urine or blood glucose.
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.[23]
5. Any cause of shortened erythrocyte survival will reduce exposure of erythrocytes to glucose with a consequent decrease in HbA1c (%) values, 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.
6. Glycated HbF is not detected by the assay as it does not contain the glycated ß-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 HbA1c values.[14,25]

Criterion: Recovery within ±10% of initial value at a decision level of 4.2% HbA1c (IFCC).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1000 µmol/L (60 mg/dL)).

Lipemia (Intralipid): No significant interference up to a triglyceride concentration of 500mg/dL(**cobas c** 501) and 400mg/dL(**cobas c** 311)

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 using common drug panels.[26]
Other: No cross reactions with HbA0, HbA1a, HbA1b, acetylated 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.

Special Wash Requirements: The use of special wash steps is necessary when certain test combinations are run together on **cobas c** systems. For information about test combinations requiring special wash steps, please refer to the latest version of the NaOHD/SMS/Multiclean method sheet and the operator manual for further instructions.

Measuring range for Whole Blood and Hemolysate Application
Hemoglobin: 4-35 g/dL
HbA1c: 0.3-2.5 g/dL
The technical limit in the instrument setting is defined as 0.9-3.1 g/dL due to the instrument factor for HbA1c (b = - 0.6; see above chapters **cobas c** 311 test definition and **cobas c** 501 test definition).
This corresponds to a measuring range of 2.3-18.9% HbA1c at a typical hemoglobin concentration of 13.2 g/dL (IFCC values; corresponding values for DCCT/NGSP: 4.3-18.9% HbA1c).

Lower detection limit
Hemoglobin: 0.5 g/dL
HbA1c: 0.1 g/dL

A typical lower detection limit for %HbA1c may be calculated, based on a given Hb concentration. Assuming a typical Hb concentration of 13.2 g/dL, the lower detection limit for %HbA1c is 0.8% (IFCC values; corresponding value acc. to DCCT/NGSP is 2.9%).

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

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Expected values for Whole Blood and Hemolysate Application

Protocol 1 (acc. to IFCC): 2.9-4.2% HbA1c[27]

Protocol 2 (acc. to DCCT/NGSP): 4.8-5.9% HbA1c[27]

HbA1c levels above the established reference range are an indication of hyperglycemia during the preceding 2 to 3 months or longer. HbA1c levels may reach 20% or higher in poorly controlled diabetes. Therapeutic action is suggested at levels above 8%. Diabetes patients with HbA1c levels below 7% meet the goal of the American Diabetes Association.[18] 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 for Whole Blood Application

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained (data based on DCCT/NGSP values):

Within-run	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
Control Level 1	5.9	0.09	1.5
Control Level 2	10.5	0.11	1.1
Human sample 1	5.2	0.04	0.8
Human sample 2	9.0	0.11	1.2

Total	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
Control Level 1	6.0	0.12	1.9
Control Level 2	10.5	0.21	2.0
Human sample 3	5.6	0.07	1.3
Human sample 4	11.8	0.20	1.7

Method comparison

HbA1c (%) values for human blood samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a COBAS INTEGRA 800 analyzer (x) and on a Roche/Hitachi MODULAR P analyzer (x).

*x = COBAS INTEGRA 800 analyzer, y = **cobas c** 501 analyzer*
Sample size (n) = 109

Passing/Bablok[28]	Linear regression
y = 0.9901x + 0.1097	y = 1.0033x + 0.0231
τ = 0.9578	r = 0.9962

The sample concentrations were between 5.0 and 12.6% (DCCT/NGSP values).

*x = Roche/Hitachi MODULAR P analyzer, y = **cobas c** 501 analyzer*
Sample size (n) = 93

Passing/Bablok[28]	Linear regression
y = 0.9837x + 0.1355	y = 0.9785x + 0.1967
τ = 0.9396	r = 0.9951

The sample concentrations were between 5.1 and 13.1% (DCCT/NGSP values).

In addition, a comparison to a commercially available HPLC method was performed. The HPLC method was standardized in conformance with DCCT (Diabetes Control and Complications Trial).[3,4]

HPLC method

Sample size (n) = 40

Passing/Bablok[28]	Linear regression
y = 0.9349x + 0.4499	y = 0.9236x + 0.5666
τ = 0.9505	r = 0.9929

The sample concentrations were between 5.3 and 11.9 % (DCCT/NGSP values).

Specific performance data for Hemolysate Application

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63). The following results were obtained (data based on DCCT/NGSP values):

Within-run	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
Control Level 1	5.9	0.13	2.2
Control Level 2	10.4	0.11	1.0
Human sample 1	5.2	0.08	1.5
Human sample 2	8.8	0.07	0.8

Total	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
Control Level 1	6.0	0.12	2.0
Control Level 2	10.1	0.17	1.6
Human sample 3	5.6	0.09	1.6
Human sample 4	11.7	0.18	1.5

Method comparison

HbA1c (%) values for human blood samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a COBAS INTEGRA 800 analyzer (x) and on a Roche/Hitachi MODULAR P analyzer (x).

*x = COBAS INTEGRA 800 analyzer, y = **cobas c** 501 analyzer*
Sample size (n) = 109

Passing/Bablok[28]	Linear regression
y = 1.0322 x - 0.1447	y = 1.0268 x - 0.1082
τ = 0.9619	r = 0.9979

The sample concentrations were between 5.2 and 13.1 % (DCCT/NGSP values).

*x = Roche/Hitachi MODULAR P analyzer, y = **cobas c** 501 analyzer*
Sample size (n) = 94

Passing/Bablok[28]	Linear regression
y = 1.0000 x + 0.0740	y = 0.9996 x + 0.1047
τ = 0.9504	r = 0.9968

The sample concentrations were between 5.1 and 13.1 % (DCCT/NGSP values).

In addition, a comparison to a commercially available HPLC method was performed. The HPLC method was standardized in conformance with DCCT (Diabetes Control and Complications Trial).[3,4]

HPLC method

Sample size (n) = 40

Passing/Bablok[28]	Linear regression
y = 0.9490x + 0.4066	y = 0.9641x + 0.3276
τ = 0.9544	r = 0.9936

The sample concentrations were between 5.3 and 11.9 % (DCCT/NGSP values).

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

The IFCC and NGSP directed the manufacturers not to change their current HbA1c report out values until further decisions by the ADA/EASD/IDF working group on the HbA1c assay.[29] This means that most countries should continue to report the established DCCT/NGSP values. If you are uncertain of the situation in your country, please contact your local authorities to ensure which approach is appropriate for your laboratory.

References

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