08038716001V2.0

cobas HbA1c Test

cobas®

Hemoglobin A1c

REF 08038694190 | Σ 10 | SYSTEM **cobas b** 101

English

Intended use

The **cobas b** 101 is an in vitro diagnostic test system designed to quantitatively determine the % hemoglobin A1c (DCCT/NGSP) and mmol/mol hemoglobin A1c (IFCC) in human capillary and venous whole blood by photometric transmission measurement. An estimated average glucose level (eAG) is calculated by the **cobas b** 101 system. The system is intended for professional use in a clinical laboratory setting, or point of care (PoC) locations.
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 (Hb) is the red-pigmented, iron-containing protein, located in the erythrocytes. Its main function is to transport oxygen and carbon dioxide in blood. Hb consists of a variety of variants (such as adult HbA and fetal HbF) and derivatives (e.g. acetylated, glycated). HbA makes up the largest fraction (> 95 %) of Hb in adult subjects and consists of 4 protein chains (2 alpha, 2 beta chains). HbA1c is one of the glycated hemoglobins, a subfraction formed by the attachment of various sugars to the HbA molecule. HbA1c is formed in two steps by the nonenzymatic 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. 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 rather than daily variations in blood glucose levels. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus.[1,2,3,4,5,6]

The risk of diabetic, microvascular 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.[7,8] 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.[1] Based on recommendations of an international expert committee[9], the WHO[10] and three diabetic associations[11,12,13] conclude that HbA1c values ≥ 6.5 % may be used to diagnose diabetes[14,15], and that % HbA1c values in the range between 5.7 and 6.4 % are suitable for identifying people who are at increased risk for developing type-2 diabetes.[16] This recommendation is based on several studies demonstrating that HbA1c concentrations ≥ 48 mmol/mol (6.5 %) were at least as strongly correlated with the development of diabetic retinopathy as blood glucose concentrations.[9,17,18]

Test principle

The blood sample is diluted and mixed with TRIS buffer to release hemoglobin from the erythrocytes. A fraction of the sample is conveyed into a reaction chamber where it is mixed with sodium lauryl sulfate (SLS). SLS is used to form the SLS-hemoglobin complex. The concentration of total hemoglobin is calculated from the measured absorbance of the SLS-hemoglobin complex at 525 nm. Hemoglobin A1c (HbA1c) in another fraction of the sample is denaturated by potassium ferricyanide and sucrose laurate. The denatured HbA1c bonds with HbA1c antibody on the latex particle. Latex agglutination is induced by the reaction of the agglutinator (which contains synthetic HbA1c epitopes) with free antibody binding sites. The concentration of HbA1c is calculated as a function of the changed absorbance measured at 625 nm which is in relation to the amount of agglutination. % hemoglobin value is calculated based on the ratio of measured concentrations of HbA1c and total hemoglobin.

Reagents

One test contains:

Dilution buffer: TRIS (hydroxymethylaminomethane): 0.94 mg

Erythrocyte Hemolysis: Sodium Lauryl Sulfate: 0.15 mg

Sodium chloride: 0.21 mg

Denaturation: Potassium ferricyanide: 60 µg, sucrose laurate: 40 µg

HbA1c antibody-latex conjugate: 85 µg

Agglutination: Glycopeptide-globulin conjugate: 2 µg

Precautions and warnings

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

Reagent handling

Carefully tear open the foil pouch at the tear notch until one side is open.

Discard the disc if the foil pouch is found open or damaged, or if the disc is damaged, or the desiccant is missing, or loose desiccant particles or any other dirt or particles especially at the blood application zone are found.

Use **cobas** HbA1c Control in the same way as a blood sample.

Storage and stability

Store at 2-30 °C until the expiration date printed on the pouch. Do not freeze. If stored in a refrigerator, allow the test to warm up in the closed pouch for at least 20 minutes before use. Once the pouch is opened, use the test within 20 minutes. Protect the disc from direct sunlight. Do not store opened pouches in a refrigerator.

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Use fresh capillary blood, lithium-heparinised or K_2 -or K_3-EDTA venous whole blood.

Do not use other anticoagulants or other additives. For EDTA samples, test within 2 hours of sample collection. For lithium-heparin samples, test within 8 hours of sample collection. Frozen whole blood samples stored at -20 °C (-4 °F) may be used up to 60 days. Freeze only one time. Mix sample thoroughly before use.

The marking on the disc clearly shows where to apply the sample. If samples are used from a venipuncture or control material, use a standard pipette or dropper to form a drop. The disc is self-filling. Do not push the sample into the disc. Do not use syringes. Assure that the disc is free from blood outside the sample application zone and the hinge cover.

Sample volume: 2 µL

Sample stability on disc

After sample application, the disc must be inserted immediately in ≤ 60 seconds. Please follow the instructions in the operator's manual.

Assay

Instructions for use

- Wash hands with soap. Warm water helps to stimulate the blood flow. Rinse the fingers extensively. Dry hands.
- Disinfect the fingertip by wiping three times the area to be lanced with a cotton swab or sterile gauze pad impregnated with 70 %-100 % isopropanol emollient free or 70 %-100 % ethanol emollient free; repeat the procedure with a second cotton swab or sterile gauze pad impregnated with 70 %-100 % isopropanol emollient free or 70 %-100 % ethanol emollient free, then dry with a cotton swab or sterile gauze pad.
- Prick the patient's finger by applying a single-use disposable lancing device (e.g. Accu-Chek Safe-T-Pro Plus). Make sure to follow the according lancing device instructions for obtaining a blood sample.
- Wipe off the first drop of blood with a swab.
- With the imprinted side of the disc facing upwards, position the disc's suction point above the drop of blood. The disc is self-filling.
- Apply blood and observe that it has filled the marked area. Check the sample volume: turn the disc on its backside. The area marked in blue has to be filled completely with blood. Do not push the blood into the disc.
- Press hinge cover down firmly to close the disc.
- Assure that the disc is free from blood outside the sample application zone and the hinge cover.
- Insert the disc into the **cobas b** 101 instrument. Close the lid.

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- The measurement starts automatically.

For more details, please refer to the **cobas b** 101 Quick Reference Guide or **cobas b** 101 Operator's Manual.

Materials provided

- REF 08038694190, **cobas** HbA1c Test, 10 tests

Materials required (but not provided)

- Single use disposable lancing device (e.g. Accu-Chek Safe-T-Pro Plus)
- REF 06380204190, **cobas** HbA1c Control
- REF 06378668190, **cobas b** 101 instrument
- Optical check disc
- General laboratory equipment (e.g., sample transfer pipette for venous blood or alcohol wipes for the fingerstick)
- Timer

Calibration

This method has been standardized against the IFCC reference method for the measurement of HbA1c in human blood[19,20] and can be transferred to results traceable to DCCT/NGSP by calculation. Each disc lot of the **cobas** HbA1c Test is traceable to IFCC.

The instrument automatically reads in the lot-specific calibration data from the barcode information printed on the disc, eliminating the need for calibration by the user.

Quality control

For quality control, use **cobas** HbA1c Control.

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.

QC info disc

Every **cobas** HbA1c Control kit contains a lot-specific QC information disc for quality control. This QC info disc contains the target values and ranges for the HbA1c Test.

The instrument display prompts the user to insert the QC info disc. The **cobas b** 101 instrument reads the disc providing the lot specific target ranges.

Display of results

At the end of the automatic determination, the **cobas b** 101 instrument shows the result in the display in less than 6 minutes. The result of the measurement will be displayed in % hemoglobin A1c (DCCT/NGSP) and mmol/mol hemoglobin A1c (IFCC).[21]

The approximate relationship between HbA1c and average blood glucose value during the preceding 2 to 3 months has been analyzed by several studies.[22] The following correlation has been established:

DCCT/NGSP standardization (% HbA1c)

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

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

To show eAG on the display, it needs to be enabled. For details, please refer to the operator's manual.

Limitations - interference

1. The test is not intended for judging day-to-day glucose control and should not be used to replace daily home testing of urine or blood glucose.
2. 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. Especially in an asymptomatic person, the diagnosis should not be made on the basis of a single abnormal plasma glucose or HbA1c value alone.[10,11]
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.[23] Whenever it is suspected that the presence of Hb variants (e. g. HbSS, HbCC or HbSC) affects the correlation between the HbA1c value and glycemic control, consider evaluation by alternative diagnostic tests, such as fasting plasma glucose (FPG) testing.
4. 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.
5. Glycated HbF is not detected by the assay as it does not contain the glycated beta-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).[24,25]
6. HbA1c results are reported for total hemoglobin concentrations of 6-20 g/dL.
7. mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) are not suitable for diagnosis of gestational diabetes.[9] In conditions associated with shortened red cell survival, such as hemolytic diseases, recent significant or chronic blood loss or after blood transfusions, the HbA1c values might not be used for diagnosis of diabetes or to monitor or manage glucose control.[26,27]
8. In very rare cases of rapidly evolving type 1 diabetes (e.g. within weeks), 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.[9]

Criterion: Recovery within ± 10 % of initial value at HbA1c concentrations in the normal and pathological range.

Icterus: No significant interference up to a conjugated/unconjugated bilirubin concentration of 1000 µmol/L or 60 mg/dL).

Lipemia (Intralipid): No significant interference up to an Intralipid concentration of 500 mg/dL. There is poor correlation between triglyceride concentration and turbidity.

Glycemia: No significant interference up to a glucose level of 111 mmol/L (2000 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.[28,29]

At physiologically occurring concentrations, no cross reactions with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin and labile HbA1c were found. The assay is specific to hemoglobin which is glycated at the beta-chain N-terminus. Consequently, the metabolic state of patients having the most frequent hemoglobinopathies (HbAS, HbAC, HbAE, HbAD) can be determined using this assay.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

20-130 mmol/mol (IFCC) or 4-14 % (DCCT/NGSP)

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Expected values

According to the recommendations of the American Diabetes Association (ADA), values above 48 mmol/mol HbA1c (IFCC) or 6.5 % HbA1c (DCCT/NGSP) are suitable for the diagnosis of diabetes mellitus. 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.[9,11] 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 53 mmol/mol HbA1c (IFCC) or 7 % HbA1c (DCCT/NGSP) in nonpregnant adults[16], and 7.5 % in children.[15,16] Lowering HbA1c to below or around 7 % has been shown to reduce microvascular and neuropathic complications of diabetes.[30] 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.

The American Diabetes Association recommends HbA1c testing 2-4 times per year for patients with diabetes. Lowering HbA1c to below or around 7 % has been shown to reduce microvascular and neuropathic complications of diabetes and, if implemented soon after the diagnosis of diabetes, is associated with long-term reduction in microvascular disease. Therefore a reasonable HbA1c goal for nonpregnant adults in general is < 7 %.[7,9,31,32]

Physicians should reevaluate the treatment regimen in patients with HbA1c values consistently > 8.0 %. Patients with an HbA1c of 5.7-6.4 % should be referred to an effective ongoing support program targeting weight loss of 7 % of body weight and increasing physical activity to at least 150 minutes per week of moderate activity such as walking. More stringent HbA1c targets might be adequate for selected patients, if this can be achieved without significant hypoglycemia or other adverse effects of treatment. HbA1c levels below the established reference range may indicate recent episodes of hypoglycemia, the presence of Hb variants, or shortened lifespan 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 instruments are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using controls in a CLSI EP5-A2 protocol. Whole blood samples were measured using a modified CLSI protocol in 5 series of 4 replicates in one day. The following results were obtained:

	Mean Value	Repeatability		Intermediate Precision	
Sample	**% HbA1c**	**SD***	% CV*	**SD***	**% CV***
Control level 1 (n[a)] = 84)	5.6	-	1.7	-	1.9
Control level 2 (n = 84)	9.9	-	1.3	-	2.0
EDTA whole blood 1 (n = 20)	5.5	-	0.8	-	1.0
EDTA whole blood 2 (n = 20)	6.7	-	1.3	-	1.5
EDTA whole blood 3 (n = 20)	8.1	-	1.4	-	1.4
EDTA whole blood 4 (n = 20)	11.5	-	1.5	-	1.8

a) n = no. of samples

* According to the set acceptance criteria, either SD or CV is shown

Method comparison

A comparison of results was obtained with 3 different lots of the **cobas b** 101 HbA1c test on the **cobas b** 101 instrument with the **cobas c** 501 analyzer using Tina-quant Hemoglobin-A1c-Gen.-3-reagent. Measurements were performed by using capillary blood on the **cobas b** 101 instruments and EDTA whole blood samples on the **cobas c** 501. A representative lot showed the following result.

Sample size (n) = 62

Mean difference = 0.19 % HbA1c

95 % of all differences obtained were between - 0.24 % HbA1c to + 0.62 % HbA1c.

The sample concentrations were between 4.7 and 9.3 % HbA1c.

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For further information, please refer to the appropriate Operator’s Manual for the instrument concerned, and the Method Sheets of all necessary components.

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 dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE

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