cobas HbA1c Test

Hemoglobin A1c

REF 06378676119

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. Measurement of hemoglobin A1c is used for diabetes diagnosis and to monitor long term blood glucose control.

Note
Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit catalogue number: 06378676190 for the cobas HbA1c test. The last 3 digits -190 have been replaced by -119 for logistic purposes.

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]

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 by measuring SLS-hemoglobin complex with a wavelength of 525 nm. Hemoglobin A1c (HbA1c) in another fraction of the sample is first denaturated by potassium ferricyanide and sucrose laurate. The denatured HbA1c bonds with HbA1c antibody on the latex particle. Latex agglutination inhibition reaction then occurs by reacting the agglutinator that has synthetic antigen which can bond with HbA1c antibody. The concentration of HbA1c is calculated by measuring the latex agglutination inhibition reaction with a wavelength of 625 nm.
% hemoglobin A1c value is measured using a ratio of concentrations of HbA1c to 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.

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, K_2 or K_3-EDTA venous whole blood only.

Do not use other anticoagulants or other additives. 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.

Materials provided
- REF 06378676190, **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[7,8] 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).[9]

The approximate relationship between HbA1c and average blood glucose value during the preceding 2 to 3 months has been analyzed by several studies.[10] 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. 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.[11]
3. 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.
4. 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).[12,13]
5. If the concentration of hemoglobin is less than 6 g/dL or higher than 20 g/dL, no test result is reported.

Criterion: Recovery within ± 10 % of initial value at HbA1c concentration of 6 % HbA1c.

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.[14,15]

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) can be determined using this assay.

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

Measuring range

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

Expected values

In 2009, an international Expert Committee that included representatives of the ADA (American Diabetes Association), the International Diabetes Federation (IDF), and the European Association for the Study of diabetes (EASD) recommended the use of the HbA1c test to diagnose diabetes, with a threshold of ≥ 6.5 %, and ADA adopted this criterion in 2010.

The HbA1c methods used must be certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. The availability of the HbA1c result at the time a patient is seen (point of care testing) has been reported to result in increased intensification of therapy and improvement in glycemic control. HbA1c levels above the established reference range are an indication of hyperglycemia during the preceding 2 to 3 months or longer. The ADA 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 %.[16,17,18,19]

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 blood samples and controls in an internal protocol.

Intermediate precision was measured with 3 lots of HbA1c test using 4 different EDTA whole blood samples at the medical decision points and 2 control solutions over 21 days with 2 series each, samples randomized, duplicate measurements per series and specimen. Repeatability was tested with whole blood samples in series of 21 measurements per specimen. The following results were obtained:

	Repeatability			**Intermediate precision**		
Sample	Mean % HbA1c	SD % HbA1c	CV %	Mean % HbA1c	SD % HbA1c	CV %
Sample low	5.3	0.11	2.0	4.9	0.14	2.8
Sample decision	6.2	0.12	2.0	5.2	0.11	1.7
Sample medium	8.0	0.12	1.5	7.3	0.10	1.4
Sample high	13.0	0.22	1.7	10.5	0.11	1.0
Control Level 1	5.7	0.14	2.5	5.5	0.13	2.3
Control Level 2	9.9	0.17	1.8	9.3	0.20	2.2

Method comparison

A comparison of results obtained with 3 different lots of **cobas** HbA1c Test on **cobas b** 101 instrument using heparin whole blood samples with **cobas c** 501 analyzer using the Tina-quant Hemoglobin A1c Gen.3 reagent was done. A representative lot showed the following result.

Sample size (n) = 108

Mean difference = 0.24 % HbA1c

Lower 95 % confidence interval of differences = -0.17 % HbA1c

Upper 95 % confidence interval of differences = 0.66 % HbA1c

The sample concentrations were between 4.2 -9.9 % HbA1c (NGSP/DCCT values).

References

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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.

SYSTEM	Analyzers/Instruments on which reagents can be used