HbA1c

Hemoglobin A1c (FIA)

infinosis™

REF: IN067701 Σ 25

Intended use
The infinosis™ HbA1c is a fluorescence immunoassay for the in vitro quantitative determination of cardiac Hemoglobin A1c (HbA1c) in Human whole blood. 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.

Summary
References[1-6]
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 Nterminal 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.

Test principle
Sandwich principle. Total duration of assay: **12 minutes**
Sample is added to the sample well of the test, then the fluorescence-labeled detector HbA1c antibody binds to HbA1c antigen in blood specimen. As the sample mixture migrates on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and HbA1c are captured to HbA1c antibody that has been immobilized on test strip. The more HbA1c antigen is in blood specimen, the more complexes are accumulated on test strip. Signal intensity of fluorescence of detector antibody reflects the amount of HbA1c captured. % hemoglobin A1c value is measured using a ratio of concentrations of HbA1c to total hemoglobin by instrument for infinosis™ tests.

Reagents
Materials provided
- **Test Cartridge**, 25 pcs, individually packaged
- **ID Chip**, 1 pcs
- **Sample Buffer,** 25 tubes
- **IFU**, 1 copy

Materials required (but not provided)
- infinosis™ 2020 FIA analyzer
- DiaSino incubator chamber
- Single use disposable lancing device
- Transfer pipette set (100 µL size)
- Alcohol pads
- Timer

Precautions and warnings
- For in vitro diagnostic use only.
- Carefully follow the instructions and procedures described in this instructions before testing.
- The Test cartridge should remain in its original sealed pouch until ready to use. Do not use it if the pouch is damaged or the seal is broken.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different Lots.
- Don't use Test cartridge if its Lot does not match with ID Chip that is inserted onto the instrument.
- The Infinosis™ HbA1c assay should be used only in conjunction with the instrument for infinosis™ tests.
- The tests should be applied by professionally trained staff working in certified laboratories at some remove from the patient and clinic at which the sample is taken by qualified medical personnel.
- infinosis™ HbA1c assay is single use only. Do not re-use it.
- The Test cartridge and Analyzer should be used away from vibration and magnetic field. During normal usage, the Test cartridge may introduce minute vibration, which should be regarded as normal.
- Use separate clean pipette tips and detector buffer vials for different specimens. The pipette tips and detector buffer vials should be used for one specimen only.
- Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.
- Blood specimens, used Test cartridges, pipette tips and detector buffer vials are potentially infectious. Proper laboratory safety techniques, handing and disposal methods should be followed in accordance with standard procedures and relevant regulations observed by microbiological hazard materials.
- The results should be interpreted by the physician along with clinical findings and other laboratory test results.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

Storage and stability
- Store the test kit at 2-30℃, the stability is up to the expiration date printed on package.
- Test cartridge and sample buffer should be used within 1 hour after opening the pack.

Specimen collection and preparation
- The test can be performed with **Human whole blood** only.
- Collect samples in accordance with correct medical practices.
- Use fresh capillary blood, lithium-heparinised, K2 or K3-EDTA venous whole blood only.
- It is recommended to test the sample within 24 hours after collection. Samples may be stored for up to a week at 2-8℃ prior to being tested. If testing will be delayed more than a week, samples should be frozen at -70℃ or below. Samples stored frozen at -70℃ or below for 3 months showed no performance difference. Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

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 ID chip lot of the Infinosis™ HbA1c Test is traceable to IFCC.
The instrument automatically reads in the lot-specific calibration data from the QR code information printed on the Test cartridge, eliminating the need for calibration by the user.

Quality control
- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with infinosis™ tests. For more information regarding obtaining the control materials, contact DiaSino Laboratories Co., Ltd for assistance.

Display of results
At the end of the automatic determination, instrument for infinosis™ tests shows the result in the display in 5 seconds. The result of the measurement will be displayed in % hemoglobin A1c (DCCT/NGSP). The approximate relationship between HbA1c and average blood glucose value during the preceding 4 months has been analyzed by several studies.[9,10] 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

Test setup
- Ensure that the lot number of the cartridge matches that of the sample buffer, and the ID Chip.
- If the sealed cartridge and sample buffer have been stored in refrigerator, place them at room temperature (18-25 ℃) at least 30 minutes before measurement.
- Turn on the instrument for infinosis™ tests.

Test procedure
1. Insert ID Chip into the instrument for infinosis™ tests and read ID chip information.
2. Using a pipette to transfer **5 µL** of sample **(Human whole blood)** to the **sample buffer tube** provided in the kit.
3. Close the lid of the sample mixing tube and mix the sample thoroughly for **5-10 seconds** by tapping or inverting the tube, then leave it at **room temperature** (18-25℃) for **2 minutes.**
4. Pipette out **100 µL** of **sample mixture** and load it onto the sample well on the cartridge.
5. Leave the sample-loaded cartridge at **30℃ for 10 minutes**. The DiaSino incubator chamber is suggested to use.
6. Insert the sample-loaded cartridge into the cartridge holder of instrument for infinosis™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder.
7. Press **"Test"** button on the instrument for infinosis™ tests.
8. Instrument for infinosis™ tests will start scanning the sample-loaded cartridge immediately.
9. Read the test result on the display screen of the instrument for infinosis™ tests.
10. Print out the testing results when press **"Print"** button on the instrument for infinosis™ tests.

Limitations - interference
- This test has been developed for testing human whole blood specimen only.
- 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.
- 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]

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- 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 timeaveraged 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.
- 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]
- Criterion: Recovery within ± 10 % of initial value at HbA1c concentration of 5.8 % 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
2%-15% (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 2%. Values above the measuring range are reported as > 15%.

Lower detection limit
2%
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n = 21).

Expected values
- The reference value
  NGSP(%): 4.5-6.5%
- The working range
  NGSP(%): 2-15%

NGSP: National Glycohemoglobin Standardization Program
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 are given below. Results obtained in individual laboratories may differ.

Precision
Intra-assay
Determined by by using 10 replicates from same batch to test with 4.0% HbA1c control. CV ≤ 10%.
Inter-assay
Determined by using 3 replicates from random 3 continuous batches to test with 4.0% HbA1c control CV ≤ 15%.

Linearity
A serial concentration of HbA1c controls at 2%, 5%, 7.5%,10%,12.5% and 15% were tested, the Correlation Coefficient is r ≥ 0.9918.

Method comparison
A comparison of the infinosis™ HbA1c assay (y) with the Elecsys cobas HbA1c assay (x) using 171 clinical samples gave the following correlations:

Linear regression
y = 0.986X - 0.0182
r = 0.9982

References

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Symbols

IVD In vitro diagnostic medical device	Temperature limit	Consult instructions for use	REF Catalog number
LOT Batch code	Date of manufacture	Use-by date	Contains sufficient for <n> tests
Manufacturer	Do not re-use	Do not use if package is damaged and consult instructions for use	
CE European Conformity	EC REP Authorized representative in the European Community		

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