Hemoglobin A1c (HbA1c)

Invitro Diagnostic reagent kit for quantitative determination of HbA1c/ Total Hemoglobin ratio in human whole blood samples on Photometric System.

Order Information

Cat. No.	Kit Configuration
OAR1094	Reagent 1: 2 x 20 mL Reagent 2: 1 x 14 mL Reagent 3: 3 x 50 mL Calibrator: 4 x 0.5 mL

Summary

The concentration of HbA1c is representative of the mean blood glucose level over the preceding four to eight weeks. HbA1c in the blood of diabetic patients increases with rising blood glucose levels. Therefore it is described as a long term indicator of diabetic control unlike blood glucose which is only a short term indicator of diabetic control. HbA1c test can screen for and diagnose diabetes or risk of developing diabetes. It helps to evaluate how well the person's glucose levels have been controlled by treatment over time. This test is also used as a measure of risk for the development of gestational diabetes and diabetes complications.

Principle

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse anti-human HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The measured absorbance is proportional to the HbA1c absorbed on to the surface of latex particals, which in turn is proportional to the percentage of HbA1c in the sample.

Method

Latex Immunoturbidimetric

Reagents

Storage Instructions and Reagent Stability

Reagent and Calibrator are stable up to the end of the indicated month of expiry, if stored at 2° – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!
Reagent 1: Buffered Latex
Solution Reagent 2: Buffered
Antibody Solution Reagent 3:
Lyse Solution
Calibrator: HbA1c Calibrator

Composition

Reagent contained: Buffer Solution, Latex particles, mouse anti- human HbA1c antibody, Goat anti-mouse IgG antibody, & Preservative.
Calibrator: HbA1c Calibrator (Lyo.) (Value on Label)

Warnings and Precautions

1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give falsified results.
3. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Reagents are ready to use.
HbA1c Calibrators are in lyophilized form and need to be reconstituted with 0.5 mL of distilled water before use. Calibrator after reconstitution is stable till expiry if stored at 2° – 8°C, protected from light and contamination is avoided.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Whole blood samples (EDTA) can be used. Hemolyzed blood samples must not be used.
Anticoagulant Blood samples are stable for 10 days if stored at 2˚C- 8˚C.

Sample Preparation

To determine HbA1c, hemolysates must be prepared for each sample.
1. Dispense 500 µL Reagent 3 (Lyse solution) into the tubes.
2. Add 10 µL of well mixed whole blood sample into it and mix.
3. Allow to stand for 5 minutes or until complete lysis is evident. Hemolysate samples may be stored upto 7 days at 2˚C-8˚C.

Assay Procedure

Wavelength	660 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against water blank

	Sample/Calibrator/Control
Sample	8 µL
Reagent 1	300 µL
Mix, incubate for 5 min at 37°C the add Reagent 2.	
Reagent 2	100 µL
Mix well, incubate for 30 seconds and read the absorbance A1; Incubate again for 270 seconds 37°C and read absorbance A2. Calculate ∆A = A2 – A1.	

Calculation

Use the provided calibrators for calibration.
Plot the absorbance values (A) obtained against the HbA1c concentration of each calibrator. HbA1c concentration in the sample is calculated by interpolation of its value (A) in the calibration curve.

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples. Each laboratory should establish corrective action in case of deviations in control recovery.

Performance Characteristics Measuring Range

The test has been developed determine HbA1c within a measuring range from 2 – 16%. If such value is exceeded the sample should be diluted 1 + 1 with NaCl solution (9 g/L) and results multiplied by 2.

Interferences

No interference was observed by, Ascorbic Acid up to 30 mg/dL, Bilirubin upto 40 mg/dL and triglycerides up to 1000 mg/dL

Sensitivity/Limit of Detection

The lower limit of detection is 2%.

Linearity

The higher limit of detection is 16%.

Precision

Intra-assay precision n = 20	Mean [%]	SD [%]	CV [%]
Sample 1	4.18	0.16	3.79
Sample 2	6.57	0.17	2.57

Inter-assay precision n = 20	Mean [%]	SD [%]	CV [%]
Sample 1	5.45	0.16	2.95
Sample 2	7.40	0.15	2.08

Method Comparison

A comparison of Nucleus Diagnosys HbA1c (y) with a commercially available test (x) using 15 samples gave following results:
$y = 0.939x + 0.383$; $r^2 = 0.941$

Reference Range

Normal Range: 4 % – 6 %
Each laboratory should check if the references range are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. **Goldstein DE, Little RR, Lorenz RA, et al. Tests of glycemia in diabetes. Diabetes Care 1995; 18:896-909.**
2. **The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993; 329:977-986.**
3. **American Diabetes Association, "Standards of Medical Care for Patients with Diabetes Mellitus", (Position statement), Diabetes Care, 21 (Suppl.1): S23 S31 (1998).**
4. **Pantheghini M, John WG on behalf of the IFCC Scientific Division. Implementation of haemoglobin A1c results traceable to the IFCC reference system: the way forward. Clin Chem Lab Med 2007; 45(8): 942-4.**

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