HbA_1c

Glycated Hemoglobin A1c

CE

Diagnostic liquid bi-reagent for determination of HbA1c concentration. IVD.
Store at 2-8 °C

REF: HBA1-200A

Summary:

Hemoglobin A1c is formed continuously by the addition of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. It has been demonstrated that Hemoglobin A1c in diabetic subjects is elevated 2-3 fold over the levels found in normal patients. Several investigators have recommended that Hemoglobin A1c serves as an indicator of metabolic control of the diabetic patients.

Principle:

This reagent uses 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 antihuman 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 amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

Composition:

Lyse Reagent: Stabilizers, Buffers, lysing agent, water
R1: Latex - < 0, 15 %, Buffer, Stabilizers.
R2: Mouse anti-human HbA1c monoclonal antibody - < 0.06 mg/mL, goat anti-mouse IgG polyclonal antibody - < 0.09 mg/dL, Buffer, Stabilizers.

Reagent Stability And Storage:

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.
Once opened vials are stable for 30 days at +2/+8°C. On board stability is related to auto analyzers' cooling specification and carry-over values.

Preparation:

Reagents are ready for use.

Samples:

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 7 days of collection when stored refrigerated. Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.
Auto analyzer usage: Samples should be tested by stat mode (Emergency mode) to avoid precipitation.

Preparation of Hemolysate

1. Whole blood samples are taken to room temperature,
2. Blood samples are mixed in order to mix erythrocytes homogeneously,
3. Using a calibrated pipette, transfer 1000 µL Lyse solution to the sample cup,
4. 30 µL of homogenized blood sample is transferred to the sample cup with Lyse added,
5. Hemolysate is mixed thoroughly, incubated for 5 minutes at room temperature,
6. Hemolysate is ready for use for HbA1c.

Procedure:

1. Assay conditions:
Wavelength: . . . . . 660 (600/660) nm
Cuvette: . . . . . 1 cm. light path
Temperature . . . . . 37°C

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

R1 (µL)	360
Sample (µL)	10

4. Mix and incubate 5 minutes.

5. Pipete into the cuvette:

R2 (µL)	120

6. Mix and read the absorbance after 5 minutes (A) of the R2 addition.

Calculations:

HbA1c concentration (%)
Plot (A) obtained against the HbA1c concentration of each calibrator (1 to 4 Level). HbA1c percentage in the sample is calculated by interpolation of its absorbance (A) in the calibration curve.

Quality control:

All clinical laboratories should establish an Internal Quality Control program. Check instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At pre-established intervals following the Q.C. Laboratory recommandations.

The International Federation of Clinical Chemistry (IFCC) values are calculated according to the formula given:

Conversion formula:
NGSP%= [0.09148 x (IFCC)] + 2.152

Reference Intervals (Normal Values):

Expected Values: %4.5 - 6.5 (NGSP/DCCT)
Expected Values: 26 - 48 mmol/mol (IFCC)
Levels above 6.5% HbA1c are suitable for the diagnosis of diabetes mellitus according to the data provided by NGSP. Patients with levels between 39-46 mmol/mol (IFCC) or 5.7% - 6.4% HbA1c (NGSP) have a possibility of developing diabetes risk. It is recommended that each laboratory establish its own normal range.
Reference interval has been verified by using CLSI EP28-A3c protocol.

Limitations:
- The linearity of the assay is up to 15% HbA1c. Samples with values above 15% should not be diluted and retested. Instead the values should be reported as higher than 16% (>16%).
- It has been observed that the patients who have alcoholism, high dose of acetyl salicylic acide, opiate and lead poisoning may lead to inconsistency.
- The assay is formulated for use with human whole blood samples in EDTA.
- Elevated levels of HbF may lead to insufficient evaluation of HbA1c and uremia does not interfere with HbA1c determination by immunoassay.

Performance characteristics:

The following values were obtained by comparing Sorachim reagent to a commercially available HPLC method.

Whole blood application	
n	100
Slope	1.001
Intercept	0.027
Correlation coefficient	0.990
Range of values	5% - 14% HbA1c

Low Linearity (LOQ):
(LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%): The limit of detection is 4%.
LoQ value has been verified by using CLSI EP17-A protocol.

High Linearity:
The method is linear up to 15.0%.
Linearity may considerably vary depending on the instrument used.

Precision:

	Intra-assay			Inter-assay		
	Mean (%)	%CV	n	Mean (%)	%CV	n
Low	5.46	1.45	40	5.46	2.81	40
High	10.1	1.73	40	10.1	2.72	80

Precision Studies data have been verified by using CLSI EP05-A3 protocol.

Interferences:
No significant interactions were observed for Conjugated Bilirubin, Triglycerides, Ascorbic Acid, Acetlated Hb, Carbamylated Hb up to the interferent concentration given below:
Ascorbic acid: 40 mg/dL
Total bilirubin: 48 mg/dL
Acetylated Hb: 4.8 mmol/L
Triglycerides: 2000 mg/dL
Carbamylated Hb: 7.3 mmol/L

The acceptable interference limit is set 10% below the highest interference concentration within ± 10% recovery of the target.
Interferences may affect the results due to medication or endogenous substances.
These performance characteristics have been obtained by using an analyzer. Results may vary if a different instrument or a manual procedure is used.

Disposal
Dispose the vials and contents according to the local regulations.

References:

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9. 5 International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes. Diabetes Care 2009;32(7):1327-1334.

Sorachim