GLYCOSYLATED HEMOGLOBIN (HbA1C)
(Immunoturbidimetric Method)

Intended Use

Glycated Hemoglobin A1c (HbA1C) is a reagent kit used for the determination of HbA1C in human whole blood based on Turbilatex method. The reagents are for *in-vitro* diagnostic use.

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 antihuman HbA1C monoclonal antibody is added (R2), latex-HbA1C- mouse anti human HbA1C antibody complex is formed. Agglutination is formed when goat antimouseIgG 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.

Reagent provided

1) R1-HbA1C **2**) R2-HbA1C **3)** HbA1C Calibrator Set (Optional) **4**) HbA1c Lysing Reagent

Working Reagent Preparation and Stability

Assay can be performed with use of separate R1-HbA1C reagents or with use of working reagent. For working reagent preparation, mix gently 3 parts of R1-HbA1C with 1 part of R2-HbA1C. Avoid foaming.
Stability of working reagent: 2 days at 2-8°C.

Specimen collection and preservation

The Test can be performed with human blood without special preparation of the patient. Follow standard laboratory procedures to collect specimens with EDTA.

Assay guidelines for Semi Auto Analyzers& other Fully Automated Analyzer as Required .

Reaction type	Fixed Time with Multicalibration Point
Reaction slope	Increasing
Wavelength	650 nm (600 - 660 nm)
Zero Setting	Distilled Water
Incubation time	---
Delay Time	25 sec
Reading Time	180 sec
No. Of Reading	1
Sample volume	15 µl
HbA1c Reagent 1	450 µl
HbA1c Reagent 2	150 µl
Calibrator concentration	Refer Calibrator Vial
Linearity	20%

Assay guidelines for Manual procedure- Step One:

1. Sample, Calibrator, Control to be lysed and mixed before adding to R2.
2. Step 1:Reconstitute Calibrator with 0.5 ml D/ waterand allow it to stand for a 15 minutes Then Take 15µlof Calibrator ,add1000 µlof Lysing reagent ,Provided in the kit
3. Follow same procedure for sample and control also to make lysate.
4. Whole blood sample is stable for 4 hr.
5. **CalibratorStability :**up to 1 month.

Reagent	Calibrator	Test (T)
Reagent 1	450 µl	450 µl
Calibrator	15 µl	-
Sample	-	15 µl
Incubate exactly 2 minutes Then add		
Reagent 2	150 µl	150 µl

Mix well and don't incubate, aspirate immediately. After adding reagent 2, please don't wait and aspirate to photometer within 5 seconds. Incubation will be started inside photometer.

Calculation

HbA1cconcentration=ΔA(T)/ΔA(C)xcalibratorconcentration

Normal Range

HbA1c in whole Blood : < 6 % Non Diabetic
6 - 7 % Glycemic Control
7 - 8 % Fair Control
>8% Poor Control

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Linearity:

2 – 20 %

Specifity/Interference

No interference detected, Bilirubin up to 0.5 g/L, ascorbic acid 0.5 g/L, Triglycerides 20 g/L, CarbamylatedHb 75 mmol/L, acetylated Hb 5.0 mmol/L

Precision

Within run CV±5%

Qualtity Control

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value sample be aliquoted,frozen and used as controls.

References

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Symbols

IVD In Vitro Diagnostics. Caution. Keep away from sun light. Date of Manufacture.
LOT Batch No. Read Instructions. Fragile. Product Expiry Date.
CONT Content. Storage Temperature. Keep Dry. Manufactured By.
REF Catalogue No.

KEE DIAGNOSTICS PVT LTD
CIN: U24231DL2004PTC128343 .
A subsidiary of KEE PHARMA LTD
A-8 . 3RD FLOOR. NARAINA INDUSTRIAL AREA, PHASE 2.
NEW DELHI-110028.
W. www.keediagnostics.com.
E. info@keediagnostics.com,
T. 011 43136000