HbA1c

(Turbilatex method)

INTRODUCTION

Glycated Hemoglobin A1c (HbA1c) is intended for Invitro quantitative determination of HbA1c in human whole blood. Hemoglobin A1c is a subtype of hemoglobin A that is formed by a non-enzymatic process that adducts glucose to the N-terminal of the hemoglobin beta chain. This process reflects the average hemoglobin exposure to glucose over an extended period and provides clinical significance in monitoring the blood glucose level. Studies have shown HbA1c in diabetic patients to be 2-3 times the levels found in normal individuals. HbA1c can be used as an indicator of metabolic control of the diabetic.

METHOD PRINCIPLE

The kit utilizes latex-enhanced immunoturbidimetry to measure the HbA1c level in human whole blood. The Kit utilizes the antibody-antigen reaction to directly measure the HbA1c level in whole blood. The first reaction, occurring after the sample is mixed with R1, consists of unspecified binding of total hemoglobin and HbA1c to the latex particles at the same rate. The second reaction occurs after the addition of R2 that contains mouse anti-human monoclonal antibody and goat anti-mouse IgG polyclonal antibody. Agglutination complexes will be formed from the interaction of the HbA1c bound to the latex particles with the respective antibodies. The agglutination can be measured as an absorbance which is proportional to the amount of HbA1c bound to the latex, and because the total hemoglobin and HbA1c bind to the latex at the same rate, the % HbA1c in total hemoglobin can be obtained from a calibration curve.

KIT CONTENTS

R1 - HbA1c Latex	1 x 30 ml	1 x15 ml
R2 - HbA1c Buffer	1 x 10 ml	1 x 5 ml
R3 - Lysing Reagent	1 x 50 ml	1 x 25 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 10 days on board the analyser at 2-10°C. Protect from light and avoid contamination.

The calibrators are lyopholized form contains 4 levels, the concentration and reconstitution instructions to be followed as per the volume mentioned on the calibrator vial labels.

WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate R1-HbA1c and R2-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

CONCENTRATIONS IN THE TEST

R1 - Latex 0.1%; Glycine buffer, pH 3.0, 15mmol/L

R2 - goat anti-mouse IgG polyclonal antibody 0.8mg/dl; Mouse anti-human HbA1c monoclonal antibody 0.05 mg/ml; Glycine buffer, pH 3.0, 60mmol/L

R3 - H2O2, stabilizers

WARNINGS AND NOTES

1. The Kit is for in vitro diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 630 nm
- Thermostat at 37ºC
- General laboratory equipment

SPECIMEN

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

PLOTTING OF MULTIPOINT CURVE

The Turbichem HbA1c is based on Non-Linear Reactions, hence it is strongly recommended to run Multi-standard mode to plot the Multi-point curve to have better accuracy and precise result.

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analyzers. **Programme Sheets** are available on request.

Wavelength	630 nm
Temperature	37°C
Cuvette	1 cm

HEMOSYLATE - Step One: (Only for Blood Sample)

1. Mix 500 µ l of Hemolysis Reagent with 10 µ l of well mixed whole blood for Test (T)
2. Wait for 5 minutes or until complete lysis is evident before using the sample.
3. If immediate testing is not possible, hemolysates may be stored up to 10 days at 2-8°C.

Reagent	Blank	Calibrator (C)	Test (T)
R1 HbA1C Latex	750 µl	750 µl	750 µl
Lysate Sample from Step 1	-	-	20 µl
Direct Calibrator (No need lysate)	-	20 µl	-
Distilled Water	20 µl	-	-
Mix well and incubate for 5 mins at 37° C, then add			
R2 HbA1C Buffer	250 µl	250 µl	250 µl

Mix well & incubate for 5 min. at 37 C. Measure the absorbance of calibrator & sample against reagent blank.

CALCULATION

HbA1c concentration = ΔA (T) / ΔA(C) x calibrator concentration

REFERENCE VALUES

HbA1c in whole blood	4 < 6%	Non-diabetic
	6 to 7%	Glycemic Control
	7 to 8%	Fair Control
	> 8%	Poor Control

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY 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 samples be aliquoted, frozen and used as controls

PERFORMANCE CHARACTERISTICS

- **Linearity:** 2% to 18%
- **Precision:** within Run CV ≤ 5 %
- **Specificity / Interferences**

  No interference detected for bilirubin upto 0.5 g/L ascorbic acid 0.5 g/L, triglycerides 20 g/L, carbamylated Hb 75 mmol/L and acetylated Hb 5.0 mmol/L

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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SYSTEM PARAMETERS

Method	End Point
Wavelength	630 nm
Zero Setting	Reagent Blank
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	5 mins + 5 mins
Delay Time	----
Read Time	----
No. of Reading	2
Interval Time	----
Sample Volume	0.02 ml (20 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	Refer Calibrator Vial
Units	%
Factor	----
Reaction Slope	Increasing
Linearity	18%

IVD

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CHEMLEON DIAGNOSTICS PVT LTD
64,8th Street, Tatabad,Coimbatore,
TN, India.641012.
Customer Care:+91-99948-37858
E-Mail:info@chemleon.com