Glycosylated Hemoglobin (Turbidimetric)

Reagent for in-vitro diagnostic determination of HbA1c present in human blood.

REF: V/HBT1.050	REF: V/HBT1.100

CLINICAL SIGNIFICANCE

Glycosylated Hemoglobin (GHb) is normal adult hemoglobin (HbA1) which is covalently bonded to a glucose molecule. GHb concentration is dependent on the average blood glucose concentration. It is formed progressively and irreversibly over a period of time and is stable till the life of the RBC. This process is slow, non-enzymatic and is dependent on the average blood glucose concentration over a period of time. A Single glucose determination gives a value which is true only at the time the blood sample is drawn. GHb on the other hand is unaffected by diet, insulin or exercise on the day of testing and thus reflects the average glucose level over the last several weeks. Hence, it reflects on the long term metabolic control of glucose in individuals. GHb is now widely recognized as an important test for the diagnosis of Diabetes mellitus and is a reliable indicator of the efficacy of therapy.

METHOD PRINCIPLE

In the first reaction, HbA1c interacts with anti-human hemoglobin A1c mouse monoclonal antibody-sensitized latex and in the second reaction, it will further interact with anti-human hemoglobin A1c mouse monoclonal antibody labeled-anti-mouse IgG goat polyclonal antibody. Then, measure absorbance of coagulated reaction solution and determine the ratio of HbA1c volume against total Hb amount from concentration of HbA1c and values of calibrator.

REAGENT COMPOSITION

Reagents:	Composition
R1: REAGENT 1	Latex, Buffer, stabilizers.
R2: REAGENT 2	Mouse anti-human HbA1c monoclonal antibody 0.05mg/mL, goat anti-mouse IgG polyclonal antibody 0.08mg/dL, Buffer, stabilizers.
Lysing Reagent	Lysing buffer for blood
CALIBRATOR	HbA1c concentration is stated on the vials labels

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.

  S61: avoid release in environment.

For further information, refer to the **Lab.Vie** glycosylated hemoglobin reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie reagent is stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).
Once opened the reagents are stable for 1 month if stored tightly closed at 2 - 8 ºC after use.
Calibrator: Reconstitute with 1.0 ml distilled water. Mix gently and incubate at room temperature for 10 minutes before use.
The reconstituted standard set is stable for 15 days if stored tightly closed at 2 to 8 ºC.

Deterioration
The **Lab.Vie** reagent can be damaged due to Presence of particles and turbidity.

SPECIMEN COLLECTION AND PRESERVATION

Whole Blood
Specimen preferably fresh and collected in EDTA. The stability of GHB in whole blood is reported to be for one week at 2-8°C.
1. Dispense 1 ml of Lysing Reagent into patient tubes.
2. Place 10 µl of well mixed whole blood into the appropriately labeled lysing reagent tube. Mix thoroughly.
3. Allow to stand for 5 minutes store up to 10 days 2-8°C.

SYSTEM PARAMETERS

Wavelength	630 nm
Optical path	1 cm
Assay type	Fixed time Turbidimetric
Temperature	37 °C
Zero adjustment	Distilled water

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes
- Centrifuge
- Stop watch
- Variable Micropipettes
- Automatic analyzer
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ASSAY PROCEDURE

	Calibrator	Sample
R1	225 µl	225 µl
Calibrator	10 µl	
Sample		10 µl
Mix, and incubate for 5 minutes, then add		
R2	75 µl	75 µl

Mix and read the initial absorbance after 10 seconds (A1), then after 5 minutes read the absorbance (A2).

CALCULATION

ΔA (sample or calibrator) = A2 – A1
$A = \frac{\Delta A \text{ sample}}{\Delta A \text{ calibrator}} \times \text{calibrator value}$
Calculate HbA1c % which equal to A from the table.

QUALITY CONTROL

Control is recommended to monitor the performance of manual and automated assay procedures.
Each laboratory should establish its own quality control scheme and corrective actions if controls do not meet the acceptable tolerances. For more information please contact **Lab.Vie** technical support.

Analytical sensitivity: 1.4 %
Analytical linearity: up to 14 %
Specimens showing higher concentration should be diluted 1/5 using physiological saline and repeat the assay.

INTERFERING SUBSTANCES

Conjugated bilirubin (40 mg/dL) and chyle of up to 3000 formazin unit, do not interfere.

EXPECTED VALUES

4.6% - 6.2%

REFERENCES

1. Halbert, SP. Ann. N.Y. Acad. Sci., 103, 1027:1051; 1963.
2. Klein GL, Applied Microbiology, 21:999, 1971.
3. Trivelli, L.A., Ranney, H.M., and Lai, H.T., New eng, J. Med. 284, 353(1971).
4. Bates, H.M., Lab, Mang., Vol 16 (Jan.1978).

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use		Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		