# Glycated Hemoglobin
# Latex Turbidimetry (HbA1c)

IVD CE

**PRODUCT CODE**
**TL005**

**INTENDED USE**
Quantitative determination of glycated hemoglobin (HbA1c) in human blood IVD.

**CLINICAL SIGNIFICANCE**
Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the adduction 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. In a classical study, Trivelli et al1 showed Hemoglobin AB1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that Hemoglobin A1c serve as an indicator of metabolic control of the diabetic, since Hemoglobin A1c levels approach normal values for diabetics in metabolic control.2,3,4 Hemoglobin A1c has been defined operationally as the "fast fraction" hemoglobins (Hb1A, A1B, A1c) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA0. The present procedure utilizes an antigen and antibody reaction to directly determine the concentration of the HbAB1c.

**PRINCIPLE**
This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbAB1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbAB1c monoclonal antibody is added (R2), latex HbA1c-mouse anti human HbAB1c 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 HbAB1c value is obtained from a calibration curve.

**REAGENTS**

| | |
|---|---|
| **(R1)** | Latex 0,13%, Buffer, stabilizer. |
| **(R2)** | Mouse anti-human HbA1c monoclonal antibody 0,05mg/ml, goat anti-mouse IgG polyclonal antibody 0,08mg/dl, Buffer, stabilizers |
| **(R3) (Hemolysis Reagent)** | Water and stabilizers |

**PREPARATION**
R1, R2 and R3 are ready to use. Mix gently before use.

**STORAGE AND STABILITY**
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Reagents should not be left inside the analyzer after use, they must be stored refrigerated at 2-8ºC. Latex may sediment. Mix reagents gently before use. Do not use reagents over the expiration date. R1 and R2 are stable for at least one month after opening stored at 2-8°C.
**Reagent deterioration:** Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability

**PRECAUTIONS**
All human specimens should be regarded as potentially biohazardous. Therefore, universal precautions should be used in specimen handling (gloves, lab garments, avoid aerosol production, etc.)

**SPECIMEN AND SAMPLE PREPARATION**
Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than anticoagulants are required. Collect venous blood with EDTA using aseptic technique. HbA1c in whole blood collected with EDTA is stable for one week at 2-8°C.
To determine HbA1c, a hemolysate must be prepared for each sample: 1.Dispense 1 mL Hemolysis Reagent into tubes labeled: Calibrator, Control, Patients, etc. Note: Plastic or glass tubes of appropriate size are acceptable.
2. Place 20 µL of well mixed whole blood into the appropriately labeled lyse reagent tube. Mix.
3. Allow to stand for 5 minutes or until complete lysis is evident. Hemolysates may be stored up to 10 days at 2-8°C.

**PROCEDURES**
1. Assay conditions:
   Wavelength: 660 nm (600 - 660)
   Temperature: 37ºC
   Cuvette ligth path: 1 cm
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette: (Note 2)

| | |
|---|---|
| R1 (µl) | 360 |
| Calibrator or Sample (µl) | 10 |

4. Mix and incubate 5 minutes.
5. Pippete 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**
HbA1c Control is recommended to monitor the performance of manual and automated assay procedures. Controls require hemolysis pretreatment after being reconstituted. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances

**REFERENCE VALUES:**
Recommended Values: less than 6% for a non-diabetic, less than 7% for glycemic control of a person with diabetes. Each laboratory should establish its own expected values. In using Hemoglobin A1c to monitor diabetic patients, results should be interpreted individually. That is, the patient should be monitored against him or herself. There is a (3–4) week time lag before Hemoglobin A1c reflects changes in blood glucose level

**PERFORMANCE CHARACTERISTICS**
1. **Measuring range:** From detection limit of 2% to linearity limit of 16%.
2. **Precision:**

| | **Intra-assay (n=20)** | |
|---|---|---|
| Mean (%) | 5.95 | 12.15 |
| SD | 0.19 | 0.18 |
| CV (%) | 3.20 | 1.47 |

| | **Inter-assay (n=20)** | |
|---|---|---|
| Mean (%) | 5.97 | 12.21 |
| SD | 0.14 | 0.15 |
| CV (%) | 2.31 | 1.24 |

3. **Sensitivity:** 1% = 0,056 (A)
4. **Accuracy:**
Results obtained using Bioresearch reagents (y) did not show differences when compared with another commercial reagent (x). The results obtained using 40 samples were the following: Correlation coefficient (r)2 0,995 Regression equation: y= 0,989x -0,047 The results of the performance characteristics depend on the analyzer used.

5. **INTERFERENCES**
1. Bilirubin to 50 mg/dL, ascorbic acid to 50 mg/dL, triglycerides to 2000 mg/dL, carbamylated Hb to 7,5 mmol/L and acetylated Hb to 5,0 mmol/L do not interfere in this assay.
2. It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin.
3. It has been reported that elevated levels of HbF may lead to underestimation of HA1c and, that uremia does not interfere with HbAB1c determination by immunoassay.10 It has been reported that labile intermediates (Schiff base) are not detected and therefore, do not interfere

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with HbAB1c determination by immunoassay.
4. It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
5. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

***NOTES**
1. In order to avoid contamination, it is recommended to use disposable material.
2. Use clean disposable pipette for its dispensation.

**SYMBOL ON LABELS**

| Symbols | Signify | Symbols | Signify |
|---|---|---|---|
| REF | Catalogue Number | SIZE | Pack Size |
| | Expiry Date | VOL | Volume |
| | Storage Condition | LOT | Lot Number |
| | Instruction for Use | IVD | In Vitro Diagnostics |
| | Manufacturing Date | | Manufacturer |
| | Number of Tests | 2 | For Single Use Only |
| EC REP | EC Representative | CE | European conformity |

**REFRENCES**

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