# HBA1C Turbidimetric

**HAEMOGLOBIN A1c**. Immuno-turbidimetric test. Direct determination of HbA1c without measurement of total haemoglobin.

REF **TB1030** R1: 1x30 mL + R2:1x10 mL

REF **TB1032** R1: 3x20 mL + R2:1x20 mL

## METHOD AND PRINCIPLE

Based on latex agglutination reaction. The HbA1c of the samples is absorbed on the surface of the latex particles, which subsequently react with the anti-HbA1c antibody (antigen-antibody reaction) forming agglomerates which give turbidity to the solution. This turbidity, caused by latex agglutination, is measured photometrically at 660 nm. Using a calibration curve, the concentration of HbA1c in whole blood can be calculated.

• Sample and addition of R1 (latex reagent)
• Addition of R2 (anti-HbA1c reagent) and start of reaction

| Latex with HbA1c | HbA1c Antibody complex | Antigen/antibody agglutination | Concentration Turbi Measurement |
|---|---|---|---|

## CLINICAL SIGNIFICANCE

Haemoglobin A1c (HbA1c) is a glycated haemoglobin which is formed by the non-enzymatic reaction of glucose with native haemoglobin. This process runs continuously throughout the circulatory life of the red cell (average life time 100 - 120 days). The rate of glycation is directly proportional to the concentration of glucose in the blood. The blood level of HbA1c represents the average blood glucose level over the preceding 6 to 8 weeks. Therefore, HbA1c is suitable for retrospective long-term monitoring of blood glucose concentration in individuals with diabetes mellitus. Clinical studies have shown that lowering of HbA1c level can help to prevent or delay the incidence of late diabetic complications.

As the amount of HbA1c also depends on the total quantity of haemoglobin the reported HbA1c value is indicated as a percentage of the total haemoglobin concentration.

Falsely low values (low HbA1c despite high blood glucose) may occur in people with conditions with shortened red blood cell survival (hemolytic diseases) or significant recent blood loss (higher fraction of young erythrocytes).

Falsely high values (high HbA1c despite normal blood glucose) have been reported in iron deficiency anemia (high proportion of old erythrocytes).

## REAGENT COMPOSITION

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| **Reagent (R1):** | |
| Latex | 25 mmol/L |
| Buffer | 15 mmol/L |
| **Reagent (R2):** | |
| Buffer | 15 mmol/L |
| Anti-HbA1c monoclonal antibody | 5.6 mg/dL |
| Anti-IgG antibody | 12 mg/dL |

## REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control blood.

After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

## SPECIMEN

Whole blood collected with EDTA. Discard contaminated specimens.

Sample preparation:

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| Distilled water | 500 µL |
| Sample | 10 µL |

Mix and allow to stand for 5 minutes or until complete lysis is apparent.

Calibrator and controls are ready to use (no preparation).

Specimen stability:

Whole blood: 1 week at 2 – 8°C

Hemolysate: 10 hours at 15 - 25°C or 10 days at 2 – 8°C

## PROCEDURE

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| Wavelength: | 660 nm |
| Temperature: | +37°C |
| Measurement: | Fixed Time against reagent blank |

Pipette as follow:

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| Reagent (R1) | 300 µL |
| Sample / Calibrator | 8 µL |

Mix, wait 120" and then add:

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| Reagent (R2) | 100 µL |

Mix, after 20 seconds read Absorbance (Abs1), after other 220 seconds read again the Absorbance (Abs2). Calculate ΔAbs (Abs2 – Abs1) for samples and calibrators.

## CALIBRATION

Results will depend on the accuracy of the instrument calibration, assay settings, the reagent/specimen ratio and the temperature control.

Calibration curve: 5 calibrators, including a 0.0 % calibrator.

Calibrate with each change of lot or if the quality control provides incorrect results.

Use MTD Diagnostics Calibrators:

**TUC1020 HbA1c Calibration Set – 5x1 mL**

Conversion Factor:

HBA1c % = (HBA1c mmol/mol / 10,929) + 2,15

HBA1c mmol/l = (HBA1c % - 2,15) x 10,929

Standardizzation:

IFCC (International Federation of Clinical Chemistry) = mmol/mol

NGSP ( National Glycohaemoglobin Standardization Program) e

DCCT (Diabete Control and Complications Trial) = %

## CALCULATION

For each sample, plot the different ΔAbs absorbances against the concentration of each calibrator dilution. Concentration in the sample is calculated by interpolation of its ΔAbs value in the calibration curve. For automatic calculation, use the SPLINE curve but other mathematical method can be used (Point-Point; Logit-Log 4P, etc..).

## QUALITY CONTROL

Normal and pathological control blood of known concentrations must be analyzed regularly in each analytical session.

The range of the values of the controls must be evaluated as a guideline, since it can be determined by the application of the method or by the user's manual skills or by other factors. The values obtained must be used for the evaluation of the Precision of the method (Repeatability). For the evaluation of the Accuracy of the method (Reproducibility) it is necessary to adhere to a program of External Quality Assessment (EQA) managed by certified bodies.

Use MTD Diagnostics controls:

**TUC1010 HbA1c Control Set 2 levels – 2x1 mL**

## EXPECTED VALUES

Non-diabetic: 20.22 - 42.08 mmol / mol (4 - 6%)
Diabetics with good compensation: 42.08 - 53.01 mmol / mol (6 - 7%)
Decompensated diabetics: 53.01 - 63.93 mmol / mol (7 - 8%)
Change Therapy:> 63.93 mmol / mol (8%)

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

## SPECIFICITY / INTERFERENCES

No interference was observed by Bilirubin up to 50 mg/dL, Ascorbic Acid up to 60 mg/dL, Lipemia (Intra-lipid) up to a triglyceride concentration of 2000 mg/dL, RF up to 250 IU/mL, Carbamylated Hb up to 7.5 mmol/L, Acetylated Hb up to 5.0 mmol/L., Uremia, HbC, Haemoglobin variants HbS and HbA2. Elevated levels of HbF may lead to falsely low HbA1c values. Alcoholism and ingestion of large doses of aspirin may lead to inconsistent results.

## PERFORMANCE CHARACTERISTICS

PRECISION:
Low Level: Samples= 20; Average = 34.43; S.D. = 0.5; CV = 1.45%
Medium Level: Samples= 20; Average 64.70; S.D. = 1.11; CV = 1.71%
High Level: Samples = 20; Average = 101.09; S.D. = 1,98; CV = 1.95%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

**y = 1.083 x – 0.560 ; r = 0.987.**

SENSITIVITY: 9,29mmol/L (3 %)

LINEARITY: 9,29 – 151,37 mmol/L (3 – 16%)

APPLICABILITY: The test is applicable to all samples with total haemoglobin included in the range 6 - 26 g / dL

## NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
3. Washing cuvette. Some instruments require, through their application protocol, a more efficient washing of the cuvettes, especially when using latex reagents. In these cases, always enter the highest number of washes foreseeable by the instrumental software.

## PRECAUTIONS

The antibody present in the preparation are of animal origin or monoclonal and are not capable of transmitting infectious diseases to humans. However, since there are no methods to ensure the total absence of such infectious agents or of other microbes, this product must be handled as if it were risky and potentially capable of transmitting infectious diseases of any kind, in accordance with Good Laboratory Practice standards.

The products do not contain dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.

However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:

S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.

S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.

S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.

S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.

S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

## SIMBOLOGY

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| CE | CE Mark (EC Directive 98/79) | | |
| IVD | In Vitro Diagnostic |  | Temperature Limitation |
|  | Consult instructions for use |  | Contains sufficient for <n> test |
| REF | Catalog Number |  | Use By |
| LOT | Batch Code |  | Manufacturer |

## BIBLIOGRAPHY

Jeppsson JO, Kobold U, Barr J, Finke A et al. Approved IFCC reference method for the measurement of HbA1c in human blood. ClinChem Lab Med 2002;40:78-89.