# HbA1c *v.2*

**Turbidimetric inhibition immunoassay for quantitative determination of HbA1c**


## SUMMARY

Diabetes mellitus is a chronic disease, comprising a group of carbohydrates metabolism disorders that are related to a common manifestation: hyperglycemia. Periodic monitoring of blood glucose levels prevents the occurrence of acute disorders and reduces the risk of long-term complications of the disease (retinopathy, neuropathy, nephropathy and cardiovascular diseases).

The relationship between the development and progression of microvascular complications and glycemic control has long been debated, partly due to inadequate methods to perform a retrospective glycemic control. The usual methods to measure glucose in blood and in urine employed to monitoring the disease have limited value for this purpose. The development of methods to determine glycosylated or glycated proteins leads to an accurate knowledge of the long-term glycemic state.

Glycohemoglobins also called glycosylated or glycated hemoglobins, were first described by Rahbar in 1968 as "diabetic hemoglobins". The glycated hemoglobins production depends on blood glucose concentration and they are formed by the non-enzymatic mechanism called glycation, where glucose is attached to the amino groups of the hemoglobin (Hb). The glycation of the N-terminal amino acids of the hemoglobin a and b chains as well as the e amino groups of lysine residues in hemoglobin molecules, result in a variety of glycated hemoglobins, including HbA1c. HbA1c is formed by glycation of free amino groups at the N-terminal amino acid valine of the hemoglobin β-chain.

HbA1c levels are proportional to blood glucose concentration during the last 6-8 weeks. Therefore, HbA1c determination provides reliable information for long-term glycemia monitoring in the diabetic patient.


## PRINCIPLE

**HbA1c v.2 Turbitest AA** is a turbidimetric inhibition immunoassay to determine hemoglobin concentration A1c (HbA1c) as a percentage of a total hemoglobin in human whole blood (% or mmol/mol HbA1c). Consequently, the hemoglobin contained in red blood cells is released by hemolysis of the sample.

**Reactivo Hemolizante** (Hemolyzing Reagent) containing a detergent (tetradecyltrimethylammonium bromide - TTAB) to specifically lysate red blood cells is added to the patient's blood. HbA1c and Hb levels in the sample are determined from the obtained hemolysate by two independent reactions.

### HbA1c

During the first stage of the reaction, the HbA1c in the sample reacts with the anti-HbA1c specific antibody (Reagent $A_1$) to form soluble antigen-antibody complexes. Since the HbA1c molecule has only one epitope per b-globin for specific antibody binding, immune complex formation does not take place.

Then, the polyhapten is added (Reagent $A_2$), which has numerous epitopes per molecule and reacts with the specific antibody excess from the first reaction, producing insoluble immune complexes which can be measured turbidimetrically at 340 nm. Therefore, the greater the HbA1c content in the sample, the lesser is the insoluble immune complex formation and the lesser the obtained turbidimetric signal.

### Hb

Released hemoglobin in the hemolyzed sample is converted to a derivate that can be spectrophotometrically measured (Reagent B).

This method is capable of detecting all hemoglobin variants which are glycated at the N-terminal of the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c. Thus, the metabolic state of diabetic patients having hemoglobinopathies and uremia can be monitored using this assay.

## PROVIDED REAGENTS

**$A_1$. Reagent $A_1$:** monospecific antibodies anti-HbA1c in pH 6.2 buffer.
**$A_2$. Reagent $A_2$:** polyhapten-HbA1c in pH 6.2 buffer.
**B. Reagent B:** pH 7.4 phosphate buffer.

## NON-PROVIDED REAGENTS

- Wiener lab's **HbA1c Calibrator Turbitest AA**
- Wiener lab's **Reactivo Hemolizante**.
- Demineralized water.
- Saline solution.

## INSTRUCTIONS FOR USE

**Reagents $A_1$, $A_2$** and **B:** ready to use.
**Reactivo Hemolizante:** ready to use.
**HbA1c Calibrator Turbitest AA:** refer to the reconstitution process in the corresponding package insert. The calibrator does not require pretreatment with **Reactivo Hemolizante**.

## WARNINGS

Provided Reagents are for "in vitro" diagnostic use.
The TTAB detergent is corrosive. H314 Causes severe skin

burns and eye damage. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P280 Wear protective gloves/protective clothing/eye protection/face protection.
Use the reagents according to the working procedures for clinical laboratories.
All reagents and samples should be discarded according to the local regulations in force.

**STABILITY AND STORAGE INSTRUCTIONS**
The **Provided Reagents** are stable in refrigerator (2-10ºC) until the expiration date stated on the box. Do not freeze.
**Reagents $A_1$, $A_2$** and **B:** once opened the reagents are stable for one month in refrigerator (2-10ºC). Keep the reagents in refrigerator and tightly closed when unused to avoid frequent recalibrations. Do not freeze.
**Reactivo Hemolizante:** stable in refrigerator (2-10ºC) until the expiration date stated on the box. Avoid contamination (do not introduce pipettes or other elements) and close the bottle after use.

**SAMPLE**
Anticoagulated whole blood
**a) Collection:** obtain in the usual way.
**b) Additives:** use heparin or EDTA (Wiener lab's **Anticoagulante W**) as anticoagulant.
**c) Known interfering substances:** no interferences have been observed by bilirubin (conjugated and non-conjugated) up to 40 mg/dl, triglycerides up to 13 g/l, ascorbic acid up to 50 mg/dl and rheumatoid factor up to 500 U/ml.
Mind the HbA1c values obtained in those pathologies and situations that alter the mean life of erythrocytes, as hemolytic anemia, ferropenic anemia, transfusions, blood loss, etc.
See Young, D.S. in References for effect of drugs on the present method.
**d) Stability and storage instructions:** the sample is stable for 3 days at room temperature (15-25ºC), for 7 days refrigerated (2-10ºC) or for 6 months frozen (-20ºC). Samples can only be frozen and thawed once.

**SAMPLE PREPARATION**
Bring **Reactivo Hemolizante** to room temperature prior to use. Homogenize the blood sample by repeated inversion, avoiding foam formation.
In a Kahn or hemolysis tube, add:

| | |
|---|---|
| **Reactivo Hemolizante** | 1000 ul |
| **Sample** | 10 ul |

Mix using a vibration mixer or by gentle swirling. Avoid foam formation. The hemolyzed sample can be used after the solution has changed color from red to brownish-green (1-2 minutes).

**Stability of the hemolyzed sample:** stable for 4 hours at room temperature (15-25ºC), for 24 hours refrigerated (2-10ºC) or for 6 months frozen (-20ºC). Samples can only be frozen and thawed once.

**REQUIRED MATERIAL** (non-provided)
- Autoanalyzer.
- Micropipettes and pipettes for measuring stated volumes.
- Kahn or hemolysis tubes.

**ASSAY CONDITIONS**
**General parameters for automatic analyzers:**

| Test name | HbA1c |
|---|---|
| Reaction type | endpoint |
| Primary wavelength | 340 nm |
| Temperature | 37ºC |
| Sample volume | 10 ul |
| Reagent $A_1$ volume | 250 ul |
| Reagent $A_2$ volume | 50 ul |
| Reagent $A_1$ incubation | 300" |
| Reagent $A_2$ incubation | 300" |
| Calibration | 5 points |
| Calibrators | Calibrator dilutions: 10, 20, 40, 60 and 100%* |

*HbA1c Calibrator dilutions are performed with Reactivo Hemolizante

| Test name | Hb |
|---|---|
| Reaction type | endpoint |
| Primary wavelength | 570 nm |
| Secondary wavelength | 660 nm |
| Temperature | 37ºC |
| Sample volume | 20 ul |
| Reagent B volume | 230 ul |
| Reagent B incubation | 300" |
| Calibration | 2 points |
| Calibrators | C0* and HbA1c Calibrator |

*Use saline solution or **Reactivo Hemolizante** as "C0" (Calibrator 0).

Sample and reagent volumes may proportionally change without affecting the calculation factors.
Refer to the appropriate applications for Wiener lab autoa-nalyzers. The applications not provided by Wiener lab. must be validated.

**CALCULATIONS**
**1) According to IFCC:**

$$\text{HbA1c (mmol/mol)} = \frac{\text{HbA1c}}{\text{Hb}} \times 1000$$

**2) According to DCCT/ NGSP:**

$$\text{HbA1c (\%)} = 91.5 \times \frac{\text{HbA1c}}{\text{Hb}} + 2.15$$

We recommend the use of a decimal place to express NGSP results (%) and no decimal places for IFCC results (mmol/mol).

**QUALITY CONTROL METHOD**
HbA1c Control normal-patológico Turbitest AA.
The controls do not require pretreatment with the **Reactivo Hemolizante**.

## REFERENCE VALUES

Metabolically healthy patients:
1- HbA1c (mmol/mol): 29-42 (according to IFCC)
2- HbA1c (%): 4.8-5.9 (according to DCCT/NGSP)
Based on DCCT and UKPDS studies, the HbA1c levels above 7% (DCCT/NGSP) or 53 mmol/mol (IFCC) are related to a higher risk for chronic complications.
Each laboratory should determine its own reference range for its patient population.

## PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
The components of the HbA1c v.2 Turbitest AA kit are lot-specific; therefore they cannot be exchanged with other lots. Perform a complete recalibration when the reagent lot is changed or when determined by the QC department.
This method was designed to report HbA1c (mmol/mol) or HbA1c (%), therefore HbA1c and Hb values should be reported separately. Avoid contamination to preserve the integrity of the reagents. Use only thoroughly clean and dry micropipettes for measurements.

## PERFORMANCE

**a) Imprecision:** evaluated by protocol EP5-A from CLSI. Three samples with different HbA1c (%) levels were tested in Konelab 60i. The following results were obtained:

**Intra-assay precision**

**Hb**

| Level | S.D. | C. V. |
|---|---|---|
| 15.1 g/dl | 0.093 g/dl | 0.6% |
| 13.2 g/dl | 0.061 g/dl | 0.5% |
| 17.7 g/dl | 0.152 g/dl | 0.9% |

**HbA1c**

| | | |
|---|---|---|
| 0.58 g/dl | 0.006 g/dl | 1.1% |
| 0.68 g/dl | 0.006 g/dl | 0.9% |
| 1.23 g/dl | 0.016 g/dl | 1.3% |

**%HbA1c**

| | | |
|---|---|---|
| 5.7 % | 0.049 % | 0.9 % |
| 6.9 % | 0.054 % | 0.8 % |
| 8.5 % | 0.087 % | 1.0 % |

**Total precision**

**Hb**

| Level | S.D. | C.V. |
|---|---|---|
| 15.1 g/dl | 0.132 g/dl | 0.9% |
| 13.2 g/dl | 0.081 g/dl | 0.6% |
| 17.7 g/dl | 0.203 g/dl | 1.2% |

**HbA1c**

| | | |
|---|---|---|
| 0.58 g/dl | 0.013 g/dl | 2.2% |
| 0.68 g/dl | 0.013 g/dl | 1.9% |
| 1.23 g/dl | 0.036 g/dl | 2.9% |

**%HbA1c**

| | | |
|---|---|---|
| 5.7 % | 0.076 % | 1.3% |
| 6.9 % | 0.092 % | 1.3% |
| 8.5 % | 0.195 % | 2.3% |

**b) HbA1c measuring range:** HbA1c values may be obtained between 0.3 g/dl and the highest HbA1c calibrator concentration (2.6 g/dl), corresponding to an approximate dynamic range of 23 to 200 mmol/mol HbA1c according to IFCC and of 4.3 to 20.5% according to DCCT/NGSP, considering a normal Hb level (13 g/dl). If HbA1c concentration is below 0.3 g/dl, the original sample must be hemolyzed 1 + 50 with **Reactivo Hemolizante** and repeat HbA1c and Hb determinations, without correcting the obtained results.
If HbA1c concentration is above the highest calibrator concentration, dilute the hemolysate 1+1 or hemolyze the original sample 1 + 200 with **Reactivo Hemolizante** and repeat HbA1c and Hb determinations, without correcting the obtained results.
**c) Hb measuring range:** 6 to 30 g/dl.

## WIENER LAB. PROVIDES

1 x 50 mL Reagent $A_1$
1 x 10 mL Reagent $A_2$
1 x 50 mL Reagent B
(Cat. Nº 1453861)

2 x 50 mL Reagent $A_1$
2 x 10 mL Reagent $A_2$
2 x 50 mL Reagent B
(Cat. Nº 1009270)

2 x 52 mL Reagent $A_1$
2 x 12 mL Reagent $A_2$
2 x 52 mL Reagent B
(Cat. Nº 1009385)

2 x 60 mL Reagent $A_1$
2 x 12 mL Reagent $A_2$
2 x 60 mL Reagent B
(Cat. Nº 1009651)

Separately provided:
**Reactivo Hemolizante:**
- 1 x 500 ml (Cat. Nº 1999701)

## REFERENCES


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**SYMBOLS**

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

| | |
|---|---|
| CE | This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices |
| EC REP | Authorized representative in the European Community |
| IVD | "In vitro" diagnostic medical device |
| | Contains sufficient for <n> tests |
| | Use by |
| | Temperature limitation (store at) |
| | Do not freeze |
| | Biological risks |
| | Volume after reconstitution |
| Cont. | Contents |
| LOT | Batch code |
| | Manufactured by: |
| | Harmful |
| | Corrosive / Caustic |
| | Irritant |
| | Consult instructions for use |
| Calibr. | Calibrator |
| CONTROL | Control |
| CONTROL + | Positive Control |
| CONTROL − | Negative Control |
| REF | Catalog number |

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