



# HbA1c

*enzymatic*

Enzymatic method for HbA1c determination in whole blood

## SUMMARY

Diabetes mellitus is a chronic disease which includes a series of carbohydrate metabolism disorders that produce a common manifestation: hyperglycemia.

Monitoring blood glucose levels prevents the occurrence of acute complications 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. Traditional blood glucose and urine monitoring methods have limited value for this purpose and was only after the development of glycosylated or glycated proteins assays when an objective and accurate knowledge of the long-term glycemic state was attained.

Glycohemoglobins also called glycosylated or glycated hemoglobins, were first described by Rahbar in 1968 as "diabetic hemoglobins". Their production depends on blood glucose concentration and occurs by post-translational, non-enzymatic mechanism called glycation, wherein glucose gets attached to the amino groups of the hemoglobin (Hb) molecules. The glycation of the N-terminal amino acids of the  $\alpha$  and  $\beta$  chains as well as the  $\epsilon$ -amino groups of lysine residues in hemoglobin molecules, result in a variety of glycated hemoglobins, including HbA1c, which is glycosylated in the N-terminal valine of the  $\beta$ -chain.

%HbA1c levels are proportional to blood glucose concentration during the last 6-8 weeks. Therefore, %HbA1c determination provides much more reliable information for long-term glucose monitoring.

## PRINCIPLE

**HbA1c enzymatic** is an enzymatic method in which lysed whole blood samples are subjected to protein digestion by a protease. This process releases amino acids, including glycated valines of hemoglobin beta chains. The glycated valines act as substrates of the enzyme fructosil valine oxidase (FVO). This enzyme specifically cleaves N-terminal valines and generates hydrogen peroxide which is quantified in a reaction catalyzed by peroxidase (POD) in chromogen presence. A separate measurement of total hemoglobin (Hb) is not required.

The concentration of HbA1c is directly expressed as %HbA1c.

## PROVIDED REAGENTS

**A<sub>1</sub>. Reagent A<sub>1</sub>:** 5 mM Good's buffer, pH 7.0, 0.5% Triton X-100, 4 KU/mL proteases.

**A<sub>2</sub>. Reagent A<sub>2</sub>:** 1 mM Good's buffer pH 6.3.

**B. Reagent B:** 15 mM TRIS buffer, pH 8.0, FVO > 10 U/mL, 90 U/mL POD, 0.8 mM chromogen.

## NON-PROVIDED REAGENTS

- Wiener lab's **HbA1c enzymatic Lysis Buffer**.
- Wiener lab's **HbA1c enzymatic Control**.
- Wiener lab's **HbA1c enzymatic Calibrator**.
- Demineralized water.

## INSTRUCTIONS FOR USE

**Reagents A<sub>1</sub>, A<sub>2</sub> and B:** ready to use for analyzers capable of working with three reagents.

For analyzers that work with two reagents premix A<sub>1</sub> and A<sub>2</sub> reagents in a 7:3 ratio, respectively. Mix by inversion and let stand at 2-10°C for at least 12 hours prior to use.

**HbA1c enzymatic Lysis Buffer:** ready to use.

## WARNING

Provided Reagents are for "in vitro" diagnostic use.

A<sub>2</sub> and B Reagents are photosensitive. Store in dark place. Use the reagents according to the working procedures for clinical laboratories.

All reagents and samples should be discarded according to current regulations.

## STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable at 2-10°C until the expiration date stated on the box. Do not freeze.

**Reagents A<sub>1</sub>, A<sub>2</sub> and B:** once opened, store the reagents tightly capped at 2-10°C. Do not freeze.

**Premixed reagents A<sub>1</sub> and A<sub>2</sub>** are stable for up to 4 weeks at 2-10°C

**HbA1c enzymatic Lysis Buffer:** stable at 2-10°C until the expiration date stated on the label. Avoid contamination (do not insert pipettes or other elements). Close the bottle after use.

## SAMPLE

Anticoagulated whole blood

**a) Collection:** obtain in the usual way.

**b) Additives:** is recommended the use of EDTA (**Anti-coagulate W** from Wiener lab) as anticoagulant.

**c) Known interfering substances:** no interference is observed with conjugated and unconjugated bilirubin up to 15 mg/dL, triglycerides up to 2000 mg/dL, ascorbic acid up to 25 mg/dL, glucose up to 4000 mg/dL, uric acid up to 20 mg/dL and urea up to 100 mg/dL.

The %HbA1c values obtained should be interpreted care-

fully in those conditions or situations that alter the half-life of erythrocytes, such as hemolytic anemia, ferropenic anemia, transfusions, hemorrhage, etc.

Refer to Young, D.S. in references for drugs' effect on the present method.

**d) Stability and storage instructions:** the sample is stable for up to 3 days at room temperature (15-25°C), and for up to 14 days at 2-10°C.

#### SAMPLE PREPARATION

Before use, bring **HbA1c enzymatic Lysis Buffer** to room temperature. Homogenize the blood sample by repeated inversion until a full suspension is obtained, avoiding foam formation. In a Kahn or hemolysis tube, add:

<b>HbA1c enzymatic Lysis buffer</b>	250 uL
<b>Sample</b>	20 uL

Mix, stirring by repeated inversion or employ vortex. Avoid foaming. Incubate at room temperature (15-25°C) for at least 10 minutes to ensure complete lysis of erythrocytes. Hemolyzed sample can be used after obtaining a dark red, limpid solution, free from particles in suspension. Calibrators and controls should be processed in the same way as patient samples.

**Stability of hemolyzed samples:** stable for up to 4 hours at room temperature (<25°C).

#### REQUIRED MATERIAL (non-provided)

- Automated analyzer.
- Micropipettes and pipettes for measuring stated volumes.
- Kahn or hemolysis tube.

#### ASSAY CONDITIONS

**General parameters for automatic analyzers capable of working with 3 reagents:**

**HbA1c enzymatic** is an endpoint method and the first reading point is immediately prior to the addition of reagent B.

Test name	HbA1c enzymatic
Reaction type	Endpoint
Primary wavelength	700 nm
Secondary wavelength	800 nm
Temperature	37°C
Reagent A <sub>1</sub> volume	112 uL
Sample volume	25 uL
Incubation	120 seconds
Reagent A <sub>2</sub> volume	48 uL
Incubation	300 seconds
Reagent B volume	70 uL
Incubation	180 seconds
Calibration	2 points
Calibrators	1 and 2

**General parameters for automatic analyzers capable of working with 2 reagents:**

**HbA1c enzymatic** is an endpoint method and the first read-

ing point is immediately prior to the addition of reagent B. Premix reagents A1 and A2 as described in the reagent preparation section.

Test name	HbA1c enzymatic
Reaction type	Endpoint
Primary wavelength	700 nm
Secondary wavelength	800 nm
Temperature	37°C
Reagent A <sub>1,2</sub> volume	160 uL
Sample volume	25 uL
Incubation	300 seconds
Reagent B volume	70 uL
Incubation	180 seconds
Calibration	2 points
Calibrators	1 and 2

Sample and reagent volumes may proportionally change, without altering the calculation factors.

Request applications for the analyzers marketed by Wiener lab. The applications not provided by Wiener lab. must be validated.

#### INTERPRETATION OF RESULTS

%HbA1c values are expressed directly according to the National Glycohemoglobin Standardization Program (NGSP) and the Diabetes Control and Complications Trial (DCCT). The results according to DCCT/NGSP (%) may be converted to IFCC (%) using the following formulation:  

$$\text{IFCC (\%)} = [\text{NGSP (\%)} - 2.15] / 0.915$$

#### QUALITY CONTROL METHOD

Wiener lab's **HbA1c enzymatic Control**.

Controls require pretreatment with **HbA1c enzymatic Lysis Buffer** reagent in the same way as calibrators and patient samples.

#### REFERENCE VALUES

People with healthy metabolism, according to DCCT/NGSP: 4.8-5.9% HbA1c

Based on studies of DCCT (Diabetes Control and Complications Trial) levels higher than 7% HbA1c are considered associated to an increased risk of having chronic complications. It is generally recommended that each laboratory establish its own reference intervals for its patient population.

#### PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

It is recommended to perform a complete calibration, when changing reagent lot and/or **HbA1c enzymatic Lysis Buffer** lot, or when suggested by Quality Control. This method was designed to report %HbA1c, therefore they should not be reported separately from HbA1c and Hb values.

The HbA1c enzymatic kit should be used whenever the patient's hemoglobin level is within the range 9-21 g/dL. Do not use this method for Hb values outside this range.

To preserve reagents integrity, avoid all forms of contamination, only using perfectly clean and dry micropipettes for measurement.

#### PERFORMANCE

**a) Reproducibility:** was assessed using EP15-A protocol

from CLSI. Three samples with different HbA1c levels were processed using Wiener lab CT600i and the following results were obtained:

#### Intra-assay precision

Level	S.D.	C.V.
5.39 %	0.04 %	0.72 %
10.89 %	0.06 %	0.56 %
12.11 %	0.09 %	0.71 %

#### Total precision

Level	S.D.	C.V.
5.39 %	0.07 %	1.32 %
10.89 %	0.09 %	0.82 %
12.11 %	0.19 %	1.54 %

**b) Detection limit:** 4 % (%HbA1c).

**c) Linearity: HbA1c enzymatic** has a linear range between 4% and 14%. Samples with values above 14% should not be diluted and retest. Results should be reported as greater than 14% (> 14%).

#### WIENER LAB PROVIDES

1 x 18 mL Reagent A<sub>1</sub>  
1 x 8 mL Reagent A<sub>2</sub>  
1 x 12 mL Reagent B  
(Cat. N° 1999735)

1 x 18 mL Reagent A<sub>1</sub>  
1 x 8 mL Reagent A<sub>2</sub>  
1 x 12 mL Reagent B  
(Cat. N° 1009368)

1 x 36 mL Reagent A<sub>1</sub>  
1 x 16 mL Reagent A<sub>2</sub>  
2 x 12 mL Reagent B  
(Cat. N° 1009621)

Separately provided:

#### HbA1c enzymatic Lysis Buffer:

- 1 x 50 mL (Cat. N°1999729)

#### REFERENCES

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- Tietz Textbook of Clinical Chemistry - Burtis, C.; Ashwood, E. (5<sup>a</sup> Ed.) WB Saunders, 2001.

- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.
- Young D.S. - "Effects of Disease on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.
- CLSI: Clinical and Laboratory Standards Institute (ex-NCCLS) Protocols EP15-A, 1999 / EP17-A, 2004.

# Symbols

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



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Manufactured by:



Authorized representative in the European Community



Harmful



"In vitro" diagnostic medical device



Corrosive / Caustic



Contains sufficient for <n> tests



Irritant



Use by



Consult instructions for use



Temperature limitation (store at)



Calibrator



Do not freeze



Control



Biological risks



Positive Control



Volume after reconstitution



Negative Control



Contents



Batch code



Catalog number

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