

# HbA1c Turbiquest

Instructions for use

Ref.: **385**

**Intended use** . System for the quantitative determination of the hemoglobin A1c (HbA1c) in whole blood samples and blood cells concentrate.

**Professional use.**

**[For in vitro diagnostic use.]**

**Test principle** . All hemoglobin present in the sample bind to the surface of latex particles (Reagent 1). The addition of the monoclonal mice antibody anti-human HbA1c (Reagent 2) provides the complex formation latex-HbA1c-antibody Anti-HbA1c. A second antibody presents in the Reagent 2 (polyclonal antibody anti-mice IgG) yields agglutination of this complex. The intensity of the agglutination, measured by absorbance, is proportional to the amount of HbA1c present in the sample. HbA1c values are obtained by a calibration curve.

**Summary** . HbA1c Turbiquest Labtest is an immunoturbidimetric method easily applicable to automated equipments capable of measuring absorbance between 600 and 660 nm. The measurement is directly performed, without need to determine total hemoglobin using only one channel of the automated equipment. Besides, results are obtained by a calibration curve, with no need to performing additional calculations. All these characteristics add more safety and are more practical to the user; moreover provides more agility and speed to the analytical process. The method is certified by the National Glycohemoglobin Standardization Program (NGSP) to TFB, Inc, with traceability to the High Performance Liquid Chromatography (HPLC) method using the Diabetes Control and Complications Trial (DCCT)<sup>3</sup> study.

**Methodology** . Immunoturbidimetry

## Reagents

### 1. **[R1]** - Reagent 1 - Store between 2 - 8 °C.

Ready for use. Contains polystyrene particles and sodium azide ≤0.05 %.

### 2. **[R2]** - Reagent 2 - Store between 2 - 8 °C.

Ready for use. Contains 0.3 % pH 6.0 buffer; mouse antihuman HbA1c monoclonal antibody; mouse anti-IgG polyclonal antibody and preservative.

### 3. **[R3]** - Hemolyzing Reagent - Store at 2 - 8 °C.

Ready for use. Contains sodium azide ≤0.05 %.

The unopened reagents, when stored under the conditions indicated, are stable until the expiration date printed on the label. During handling, the reagents are subject to chemical and microbial contamination, which may cause reduced stability.

## Precautions and warnings

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

The Reagent 1 and Hemolysis Reagent contain sodium azide as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

## Materials required not provided

1. Photometer capable of measuring with accuracy the absorbance at 600 - 660 nm;

2. Calibrators Calibra HbA1c Turbiquest Ref. 386 and controls Glicotrol Ref. 303 Labtest.

## Sample

The red blood cells concentrate or whole blood can be used. The blood should be collected with EDTA or sodium fluoride. The analyte is reportedly stable for about 7 days at 2 - 8 °C and at least one year at -70 °C.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious. Therefore, follow the established Biosecurity guidelines while handling the samples.

## Interference

Concentrations of conjugated bilirubin up to 30 mg/dL; unconjugated bilirubin up to 30 mg/dL; ascorbic acid up to 50 mg/dL do not produce significant interferences.

Inconsistent results may be obtained in patients that present the following conditions: use of opiates, poisoning with lead, alcoholism, and ingestion of high amount of acetylsalicylic acid.

High level of fetal hemoglobin in the sample produces negative interference. Hemoglobin A2, Hemoglobin C and Hemoglobin S may increase the inaccuracy of the results.

The fraction pre-HbA1c (labile or instable) is not detected by the method, therefore, it does not interfere in the HbA1c determination.

See notes 1 and 2.

Applications are available for automatic systems.

**Sample preparation** . Prepare a hemolysate for each sample.

## Concentrated red blood cell

1. Centrifuging the whole blood for 2 minutes at 2000 rpm;
2. In a tube, pipette 0.5 mL of the Hemolyzing Reagent and 0.005 mL of the red blood cell concentrate;
3. Thoroughly shake them for 10 seconds and wait 5 minutes or until the complete lysis becomes evident (absence of turbidity);
4. The hemolysate can be stored for up to 10 days between 2 - 8 °C.

## Whole blood

1. In a tube, pipette 0.5 mL of the Hemolyzing Reagent and 0.010 mL of the homogenized whole blood sample;
2. Thoroughly shake them for 10 seconds and wait 5 minutes or until the complete lysis becomes evident (absence of turbidity);
3. The hemolysate can be stored for up to 10 days between 2 - 8 °C.

**Parameters for Automatic Analyzers**

Type of Reaction	End Point
Reaction Direction	Growing
λ Primary Wave	600 to 660 nm
λ Secondary Wave	800 nm
Temperature	37 °C
Calibration	5 points (Calibra HbA1c Turbiquest - Ref. 386)
Calibration Model*	Non-linear (Spline, Exponential)
Sample Volume**	4 µL
Reagent Volume 1**	150 µL
Incubation at 37 °C	300 seconds
Reagent Volume 2**	50 µL
Absorbance 1	Between 60 and 90 seconds after adding the Reagent 2
Absorbance 2	300 seconds after adding the Reagent 2

\* The definition of calibration model must be adapted to each model of equipment. If in doubt contact the Labtest Customer Service.

\*\* Volumes and reagents sampling can be modified proportionally without prejudice to the test performance, and the calculation procedure remains unchanged. In case of volume reduction, it is essential to observe the minimum volume required for the photometric reading.

## Calibration

The method is certified by the National Glycohemoglobin Standardization Program (NGSP) for TFB, Inc., with traceability to the high-performance liquid chromatography (HPLC) method used in the Diabetes Control and Complications Trial (DCC<sup>3</sup>) study.

### 5 points calibration

Use the product Calibra HbA1c Turbiquest - Ref. 386.

Point 0: use the calibrator 0.

Point 1 to 4: use the calibrators 1, 2, 3 and 4.

### Calibration interval

When the internal quality control indicates;

When using new reagent lot.

**Operating range** . The measurement operating range is from 3.0 % to 13.0 %. Result higher than 13.0 % may be reported as greater than 13.0 % or dilute the sample 1:3 using as diluent another sample with HbA1c concentration less than 6 % (50 µL of the sample hemolysate with a high concentration and 100 µL of the diluent sample hemolysate).

Determine the HbA1c concentration in the mixture, and calculate the HbA1c concentration of the high sample as follows:

$$\text{HbA1c (\%)} = [\text{HbA1c (\%)} \text{ in the mixture } X n] - [\text{HbA1c (\%)} \text{ in the diluent sample } X (n - 1)]$$

n = sample dilution factor

### Example

$$[8.3 X 3] - [4.6 X (3 - 1)] = 24.9 - 9.2 = 15.7 \%$$

**Internal quality control** . Each laboratory should establish corrective actions to be taken if values fall outside the control limits. At the same time, a defined system should be maintained to monitor the analytical variability occurring in the whole measuring system. Control materials should be used to monitor measurement uncertainties and calibration deviations. It is suggested that the specifications for the variation coefficient and the total errors are based on the Biological Variation components (BV)<sup>5</sup> or on the specifications proposed by the NGSP<sup>6</sup>.

**Expected values** . For non-diabetic individuals 4.0 % to 6.0 %.

For diabetic individuals in control of glycemia:

Children (pre-puberty age)	< 8.0 %
Children (puberty age)	< 8.5 %
Adults	< 7.0 %
Elderly	< 8.0 %

The values for diabetic individuals were determined from results of prospective and random clinical studies and correlate to lower significant risk of development of complication of *Diabetes mellitus*<sup>3,4</sup>.

## Performance characteristics<sup>7</sup>

**Recovery studies** . Two samples with HbA1c concentrations equal to 6.2 % and 9.4 % were added with amounts of the analyte, and the results obtained are as follows:

Concentration (%)				Recovery (%)
Initial	Added	Expected	Found	
6.2	0.9	7.1	7.2	101.4
9.4	1.0	10.4	10.6	101.9

Using the average error of 1.7 %, the estimated proportional systematic error is equal to 0.099 % for samples with 6.0 % concentration and 0.167 % for samples with 10.0 % concentration.

**Methods comparison** . The proposed method was compared to the HPLC method, and the following results were obtained:

	Comparative Method	Labtest Method
Number of samples	50	50
Regression equation	Labtest Method (%) = 0.9844 x Comparative + 0.039	
Correlation coefficient	0.994	

Using the regression equation, the total systematic error (bias) estimated is equal to 0.85 % and 1.18 % for samples of 5.5 and 10.2 %, respectively. This error is less than the systematic error of the desirable specification based on the BV<sup>5</sup> components which is ≤1.50 %.

**Accuracy studies** . Accuracy studies were performed using three samples with concentrations equal to 5.5 %, 7.1 % and 10.2 %.

## Repeatability - Imprecision Within Run

	N	Average (%)	SD	CV (%)
Sample 1	15	5.5	0.034	0.62
Sample 2	15	7.1	0.032	0.45
Sample 3	15	10.2	0.037	0.36

## Reproducibility - Imprecision Run-to-Run

	N	Average (%)	SD	CV (%)
Sample 1	15	5.5	0.038	0.70
Sample 2	15	7.1	0.047	0.66
Sample 3	15	10.2	0.035	0.34

The inaccuracy found complies with the desirable specification for the total inaccuracy based on the BV<sup>5</sup> components which is ≤0.93 %.

**Methodological or analytical sensitivity** . Detection limit: 0.095 %. Equivalent to 3 standard deviations (SD) obtained from 10 measurements of a sample with HbA1c concentration equal to 3.0 %.

## Notes

**1.** The water in the laboratory to prepare reagents and use in the measurements, must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be <0.1 mg/L (Type II reagent water). The water for washing must be Type III, having resistivity ≥0.1 megaohms or conductivity ≤10 microsiemens. For the final washing, use Type II reagent water.

**2.** It is suggested to consult Young DS. Effects of Drugs on Clinical Laboratory Tests, 3<sup>rd</sup> ed., Washington: AACC Press, 1990, to review physiopathological sources and drug interferences in results and methodologies.

## References

- American Diabetes Association. Diabetes Care 2015, 38 (suppl 1).
- Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry, Philadelphia: Saunders Company 1994; 980-986.
- DCCT Research Group: Diabetes Control and Complications Trial (DCCT). N Eng J Med 1993;329:977-986.
- Interdisciplinary Group of Glycated Hemoglobin - A1c Standardization. 2004. www.sbpc.org.br (comissoes).
- Sociedad Española de Bioquímica Clínica y Patología Molecular. Available on: <[http://www.seqc.es/es/Comisiones/18/9/102/Base\\_de\\_datos\\_de\\_Variacion\\_biologica\\_%7C\\_Comision\\_de\\_Calidad\\_Analitica\\_%7C\\_Comite\\_Cientifico/](http://www.seqc.es/es/Comisiones/18/9/102/Base_de_datos_de_Variacion_biologica_%7C_Comision_de_Calidad_Analitica_%7C_Comite_Cientifico/)> (accessed Feb 2015).
- Summary of NGSP Certification Categories. Available on: <<http://www.ngsp.org/news.asp>> (accessed Jan 2015).
- Labtest: Data on file.

## Presentation

Product	Reference	Contents
HbA1c Turbiquest	385-1/20	<b>R11</b> 1 X 15 mL
		<b>R12</b> 1 X 5 mL
		<b>R13</b> 1 X 65 mL
	385-1/40	<b>R11</b> 1 X 30 mL
		<b>R12</b> 1 X 10 mL
		<b>R13</b> 1 X 130 mL
HbA1c Turbiquest Labmax 560/400	385-1/40	<b>R11</b> 1 X 30 mL
		<b>R12</b> 1 X 10 mL
		<b>R13</b> 1 X 130 mL

For information on other commercial presentations, please visit [www.labtest.com.br](http://www.labtest.com.br) or contact Customer Service.

The number of tests for automatic systems depends on the programming parameters of each equipment.

See availability of applications with Customer Service.

## Customer information

### [Warranty conditions]

**Labtest Diagnóstica** warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions indicated on the label and in this insert have been followed correctly.



**Labtest Diagnóstica S.A.**

CNPJ: 16.516.296 / 0001 - 38

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Edition: August, 2015  
Revision: February, 2019  
Ref.: 050422(02)




























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# Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

	<b>Conteúdo suficiente para &lt; n &gt; testes</b> Contenido suficiente para < n > tests Contains sufficient for < n > tests		<b>Risco biológico</b> Riesgo biológico Biological risk
	<b>Data limite de utilização (aaaa-mm-dd ou mm/aaaa)</b> Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		<b>Marca CE</b> Marcado CE CE Mark
	<b>Material Calibrador</b> Material Calibrador Calibrator Material		<b>Tóxico</b> Tóxico Poison
	<b>Material Calibrador</b> Material Calibrador Calibrator Material		<b>Reagente</b> Reactivo Reagent
	<b>Limite de temperatura (conservar a)</b> Temperatura limite (conservar a) Temperature limitation (store at)		<b>Fabricado por</b> Elaborado por Manufactured by
	<b>Representante Autorizado na Comunidade Europeia</b> Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		<b>Número do lote</b> Denominación de lote Batch code
	<b>Consultar instruções de uso</b> Consultar instrucciones de uso Consult instructions for use		<b>Controle</b> Control Control
	<b>Número do catálogo</b> Número de catálogo Catalog Number		<b>Controle negativo</b> Control negativo Negative control
	<b>Adições ou alterações significativas</b> Cambios o suplementos significativos Significant additions or changes		<b>Controle positivo</b> Control positivo Positive control
	<b>Produto diagnóstico in vitro</b> Dispositivo de diagnóstico in vitro In vitro diagnostic device		<b>Controle</b> Control Control
	<b>Liofilizado</b> Liofilizado Lyophilized		<b>Corrosivo</b> Corrosivo Corrosive
	<b>Período após abertura</b> Período post-abertura Period after-opening		<b>Uso veterinário</b> Uso veterinario Veterinary use
	<b>Instalar até</b> Instalar hasta Install before		<b>Fabricado em</b> Elaborado en Manufactured on
	<b>Produto de uso único</b> Producto de un solo uso Single use product		

Ref.: 280322 |