BIOLABO
www.biolabo.fr
MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

HbA1c Turbidimetric Immunoassay

Reagent for quantitative determination of the HbA1c in the human blood

REF K2010 **R1** 1 x 30 mL **R2a** 1 x 9.5 mL **R2b** 1 x 0.5 mL **R3** 1 x 125 mL **R4** 2 x 50 mL

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
support@biolabo.fr
Latest revision: www.biolabo.fr

CE

IVD

Made in France

I: corresponds to significant modifications

INTENDED USE

This reagent is designated for professional use in laboratory (automated method).
It allows quantitative determination of the HbA1c in the human blood to evaluate glycaemic level in diabetes mellitus.

GENERALITIES (1) (2) (3) (4)

HbA1c values provide an indication of glucose levels over the preceding 4-8 weeks. A higher HbA1c value indicates poorer glycaemic control of patients with diabetes.

PRINCIPLE (5)

Photometric measurement of turbidity, corresponding to antigen-antibody reaction, by the end-point method at 600 nm to directly determine HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific adsorption rate to latex particles. When mouse anti-human HbA1c monoclonal antibody is added (vial R2) latex-HbA1c –mouse anti-human HbAc antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody.

REAGENTS

R1	**HbA1c TIA**	Latex
Latex	0,13 %	
Glycine Buffer	20 mmol/L	
Sodium Azide	0.95 g/L	
R2a	**HbA1c TIA**	Antibody
Mouse anti-human HbA1c monoclonal antibody	0,05 mg/mL	
Buffer, Stabilizers		
R2b	**HbA1c TIA**	Antibody
Goat anti-mouse polyclonal antibody	0,08 mg/dL	
Buffer, Stabilizers		
R3	**HbA1c TIA**	Hemolyzing solution
Aqueous Solution		
Sodium Azide	0.5 g/L	
R4	**HbA1c TIA**	Cleaning solution

Classification due to sodium hydroxide
Met. Corr. 1: H290 - May be corrosive to metals.
Skin Corr. 1B: H314 - Causes severe skin burns and eye damage.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310: Immediately call a poison center/doctor.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Medical analysis laboratory equipment.
2. Biochemistry Clinical Analyzer Kenza One, Kenza 240TX/ISE or Kenza 450TX/ISE

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Reagent R1, R3, R4: Ready for use
Reagent R2: pipet exactly the volume of R2b indicated in the label and transfer into vial R2a. Well recap and mix gently until complete dissolution.

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert:
Unopened,
- Until the expiry date stated on the label of the Kit.

Once opened:
- when free from contamination and stored at 2-8°C in the original vial:
  - Reagents R1, R3 and R4 are stable at least for 3 months.
  - Reagent R2 (R2a+R2b) is stable at least for 30 days.

SPECIMEN COLLECTION AND PREPARATION (6)

Fresh venous blood collected with EDTA using aseptic technique. Special preparation of the patient is unnecessary. No special additives or preservatives other than anticoagulants are required.
Hemolysate preparation (patient(s), calibrators and controls):
1. Dispense 1 mL Hemolyzing reagent (vial R3) into well labelled plastic or glass test-tubes:
2. Add 20 µL of well mixed specimen (calibrators, controls, patient(s))
3. Mix well
4. Let stand for 5 min at room temperature until complete lysis

If the test cannot be carried out on the same day, hemolysates may be stored up to 7 days at 2-8° C.
For longer storage, freeze specimen at -70° C for maximum 30 days

LIMITATIONS (5) (10) (11) (12) (13) (14) (15)

The limitations of the method are known they are related to a modified lifetime of red blood cells, physiological hemolysis or an insufficient level of total hemoglobin, which may invalidate the test result.
Inconsistent results have been reported in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin.
It has been reported that elevated levels of HbF may lead to underestimation of HbA1c. Also, it has been reported that labile intermediates (Schiff base) are not detected and therefore, do not interfere with HbA1c determination by immunoassay.
It has been determined that hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
Other rare variants of hemoglobin (HbE) have not been assessed.
For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

I CALIBRATION

- REF 22012 HbA1c Standard Set traceable to reference material from NGSP.
- Batch specific values are indicated in the certificate of analysis and on the label of each vial.
- Construct Standard curve as indicated in the application of KENZA analyser used

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

REFERENCE INTERVALS (7) (8) (9)

	HbA1c NGSP (%)	HbA1c IFCC (mmol/mol Hb)
Non-diabetic:	< 6.0 %	42
Glycaemia control of a patient with diabetes:	< 7.0 %	53

In using Hemoglobin HbA1c to monitor diabetic patients should be interpreted individually. That is, the patient should be monitored against him or herself.

There is 3-4-week time lag before HbA1c reflects changes in blood glucose levels.

Each laboratory should verify the consistency of reference ranges for the population that it serves.

PERFORMANCES

On a clinical chemistry analyzer (Hitachi 917).

Detection limit: approx. 0.43%

Linearity range: between 2.0% and 16.0%.

Above 16%, dilute the specimen with saline and re-assay considering the dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean %	4.8	7.3	10.9
S.D. %	0.06	0.08	0.16
C.V. %	1.3	1.0	1.5

Accuracy:

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean %	4.7	7.4	11.1
S.D. %	0.06	0.08	0.17
C.V. %	1.3	1.1	1.5

Analytical Sensibility: 0,073 Abs/1.0% HbA1c

Diagnostic Specificity: Monospecific

Comparison with an automated HPLC (40 specimens from 2% to 16%)

On Hitachi 917: y = 1,010 x + 0,04 r = 0,996

Interferences

Bilirubin: No interference up to 50 mg/dL.

Ascorbic Acid: No interference up to 50 mg/dL.

Triglycerides: No interference up to 2000 mg/dL.

Carbamylated Hb: No interference up to 7,5 mmol/L

Acetylated Hb: No interference up to 5,0 mmol/L.

Other substances may interfere with the result (see §Limitations)

On-board stability: Reagents are stable at least 24h.

Calibration Frequency: It is recommended to calibrate systematically.

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

QUALITY CONTROL

- REF 22013: HbA1c Control Set
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat the tests with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

I PROCEDURE

- Let stand reagents and specimens at room temperature
- Hemolysate: Lyse patient's specimen, calibrators and controls as indicated in § "Specimen Collection and Preparation"
- Before use, mix reagent R1 by gentle swirling
- Refer to specific applications of KENZA Analyzer used

Minimal Software revision required :

- KENZA 240TX/ISE : from 6.13
- KENZA 450TX/ISE : from 2.20
- KENZA ONE : from 2.04

Contact support@biolabo.fr for more details

CALCULATION

Refer to User's manual of KENZA analyzer used.

NGSP Results (%):

The analyzer provides directly final result (%)

IFCC results (mmol/mol Hb):

Use « Master equation »:IFCC (mmol/mol Hb)=(NGSP-2,15)/0,915*10

REFERENCES

(1) Trivelli.,L .A, Ranney., H. M. and Lai, H.T.New Eng. Med. 284, 353 (1971)

(2) Gonen B. and Rubenstein A. H., Diabetologia,15, 1 (1978).

(3) Gabbay K.H., Hasty K,. Breslow J. L., Ellison R.C.,Bunn H. F.,and Gallop P.M., J.Clin. Endocrinol Metab.44, 859 (1977)

(4) Bates H. M., Lab. Mang., vol 16 (Jan 1978)

(5) TIETZ N.W. Textbook of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p.798, 800.

(6) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 480-483

(7) American Diabetes Association: Clinical practice recommendations (Position Statement). Diabetes Care (Suppl.1): S33-S55 (2001)

(8) Recommandations HAS (antérieurement ANAES) relatives au "Suivi du patient diabétique de type 2 à l'exclusion du suivi des complications" (janv. 1999)

(9) Traitement médicamenteux du diabète de type 2 (Actualisation 2006): Recommendations HAS

(10) Cerellio, A. et Al., diabetologia 22, p.379 (1982)

(11) Little R. R., et al., Clin. Chem. 32, p.358-360 (1986)

(12) Fluckiger R., et al., New England J. Med. 304, p.823-827 (1981)

(13) Nathan D. M. et al. , Clin. Chem. 29 p.466-469 (1983)

(14) Engbaeck F. et al., Clin. Chem. 35 p.93-97 (1989)

(15) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-331 to 3-332

(16) Ragnar Hanas, Garry John and On behalf of the International Consensus Committee "2010 Consensus statement on the worldwide Standardization of the Hemoglobin A1C Measurement"

(17) Hoelzel W., Weykamp C., Jeppson J.O., Miedema K., Baar J. R. IFCC "Reference System for Measurement of Hemoglobin A_{1c} in Human Blood…", Clin. Chem.50:1, p.166-174 (2004)

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with