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 22010	**R1** 1 x 30 mL	**R2a** 2 x 4,75 mL	**R2b** 2 x 0,25 mL	**R3** 1 x 125 mL
REF 22011	**R1** 1 x 60 mL	**R2a** 1 x 19 mL	**R2b** 1 x 1 mL	**R3** 2 x 125 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 (manual or 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 haemoglobin 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 HbA1c 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 reagent	
Aqueous Solution Sodium Azide		0.5	g/L

These reagents are not classified as dangerous according to 1272/2008/EC regulation.

REAGENTS PREPARATION

Reagent R1, R3: Ready for use
Reagent R2: transfer the contents of vial R2b into vial R2a, recap and mix gently.

I MATERIEL REQUIRED BUT NOT PROVIDED

1. Medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer
3. REF CO4000: Solution for cleaning measuring system of analysers

SAFETY CAUTIONS

- BIOLABO reagents are designated for professional use in laboratory
- 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.

I 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.

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 and R3 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 Haemolysis 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 is evident

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 haemolysis or an insufficient level of total haemoglobin, 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*(14)*. Also, it has been reported that labiles intermediates (Schiff base) are not detected and therefore, do not interfere with HbA1c determination by immunoassay.

It has been determined that Haemoglobin variants HbA2, HbC and Other very rare variants of haemoglobin (HbE) have not been assessed.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S .

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.

REFERENCE INTERVALS (7) (8) (9)

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

In using Haemoglobin 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.

I PERFORMANCES

On a clinical chemistry analyser Hitachi 917, at 660nm, 37°C.

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 = 10	*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 = 9	*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

Sensibility:0,073 Abs/1.0% HbA1c

Specificity: Monospecific

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

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: at least 24h on board

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

CALIBRATION

- REF 22012: HbA1c Standard Set traceable to reference material from NGSP.

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

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.
Before use, mix reagent R1 by gentle swirling.

Hemolysate Preparation: Lyse patient's specimen, calibrators and controls as indicated in § "Specimen Collection and Preparation"

Manual Procedure:

Use Standard Set REF 22012 (4 different levels) to generate a Calibration Curve.
Use saline as sample to determinate zero point

Pipette into well identified test tubes:	**Blank**	**Standards**	**Assays**
Latex (R1)	700 µL	700 µL	700 µL
Saline	20 µL		
Standards (4 different levels)		20 µL	
Specimen			20 µL
Mix well. Let stand for 5 minutes at 37°C.			
Anti-HbA1c(R2a+R2b)	250 µL	250 µL	250 µL
Mix well. Incubate for exactly 5 minutes at 37°C. Read absorbance of lysed standards, lysed controls and lysed specimens at 600 nm against Blank.			

1- With Manual Procedure on Spectrophotometer, performances and stability data should be validated by user

2- Applications proposal are available on request of other analysers

CALCULATION *(16)*

Calculate ΔAbs (Abs $_{assay}$ – Abs $_{blank}$) for standards, controls and specimens.

NGSP Results (%)

Plot a Standard Curve "HbA1c (%) = f(ΔAbs)" and read the concentration of controls and patient specimens on the graph.
Results are reported as % HbA1c versus HbA.

IFCC results (mmol/mol Hb):

Use « Master equation »from 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. Text book 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