BIOLABO
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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 19,0 mL	**R2b** 1 x 1,0 mL	**R3** 2 x 125 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2) (3) (4)

Reagent for the quantitative determination of HbA1c in human blood. HbA1c determination is commonly assayed for the evaluation of glycemic control in diabetes mellitus. HbA1c values provide an indication of glucose levels over the preceding 4-8 weeks. A higher HbA1c value indicates poorer glycemic control. Do not use for the diagnosis of diabetes mellitus.
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.

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 HbAc antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody.

REAGENTS

Vial R1 **LATEX** (Concentration in the Test)

Latex	0,13	%
Glycine Buffer	20	mmol/L
Sodium Azide	0.95	g/L

Vial R2a **ANTIBODY**

Mouse anti-human HbA1c monoclonal antibody 0,05 mg/mL
Buffer, Stabilizers

Vial R2b **ANTIBODY**

Goat anti-mouse polyclonal antibody 0,08 mg/dL
Buffer, Stabilizers

Vial R3 **HAEMOLYSIS REAGENT**

Aqueous Solution Sodium Azide 0.5 g/L

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

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

STABILITY AND STORAGE

Store at 2-8° C, away from light (Do not freeze).

- Unopened reagents are stable until expiry date stated on the label.
- 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.

MATERIEL REQUIRED BUT NOT PROVIDED

1. Saline 0.9 % (zero point for calibration curve)
2. Calibrators and Controls

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
5. Measure HbA1c (%) (§ **MANUAL PROCEDURE**)

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

CALIBRATION

- HbA1c Standard Set REF 22012 traceable to reference material from NGSP. IFCC related through « Master Equation » (CALCULATION).

Use as indicated in the insert (§ **MANUAL PROCEDURE**) to generate a reference curve.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.
It is recommended to calibrate systematically.

Manufacturer | Use by | In vitro diagnostic | Temperature limitation | Catalogue number | See insert | Batch number | Store away from light | sufficient for | dilute with

QUALITY CONTROL

- REF 22013: HbA1c Control Set
- Assayed control referring to the same method.
- External quality control program.
- Linearity of the assay should be verified with a commercial linearity check set or dilutions of a high specimen, at least every 6 months

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, patient's values from that run should not be reported and following actions should be applied:

1. Repeat the test with the same control.
2. If control is still out of range, use a new vial of control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (7) (8) (9)

	HbA1c NGSP (%)	HbA1c IFCC (mmol/mol Hb)
Non-diabetic:	< 6.0 %	42
Glycemic 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.

PERFORMANCES

The performance characteristics for the HbA1c Reagents were measured on a clinical chemistry analyzer (Hitachi 917).
The within run and between day precision studies was established by testing 2 blood specimens following NCCLS EP5 Protocol

Within run	*Low level*	*High level*
Mean %	5,5	10,3
S.D. %	0,08	0,18
C.V. %	1,43	1,72
Expected CV%	< 3%	< 3%

Between run	*Low level*	*High level*
Mean %	5,5	10,3
S.D.%	0,15	0,28
C.V. %	2,77	2,68
Expected CV%	< 4%	< 4%

Detection limit: approx. 2.0%

Sensibility:. 0,073 Abs/1.0% HbA1c

Specificity: Monospecific

Comparison with an automated HPLC Procedure:using 40 human specimens between 2.0 and 16.0%

y = 1,050 x – 0,481 (Syx = 0,332)

INTERFERENCES *(5) (10) (11) (12) (13) (14) (15)*

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.

Inconsistent results have been reported in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin [(10) (11) (12) (13)].

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[(5)].

It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.

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

LINEARITY

The assay is linear between 2.0% and 16.0%.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Before use, mix by gentle swirling Latex Reagent (vial R1).

Reconstitute calibrators and controls as indicated in the insert

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

Calibration curve:

Use Standard Set REF 22012 (4 different levels) to generate a Calibration Curve.

Use saline as sample to determinate zero point

Test:

Pipette into well identified test tubes:	**Blank**	**Standards**	**Assays**
Latex (Vial 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(Reagent R2)	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.			

Notes:

- Patient specimens should always be assayed using a calibration curve.
- Application procedures on clinical chemistry analyzers are available upon request.

CALCULATION *(16)*

Calculate the result as follows:

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

NGSP Results (%)

Plot a Standard Curve "HbA1c (%) = f(ΔAbs)".

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 »:

IFCC (mmol/mol Hb)=(NGSP-2,15)/0,915*10

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