**HbA1c**

Turbidimetric Immunoassay

Reagent for quantitative determination of the HbA1c in the human blood

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**TECHNICAL SUPPORT AND ORDERS**

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**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 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.

**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**

<table>
<thead>
<tr>
<th>Vial R1</th>
<th>LATEX</th>
<th>(Concentration in the Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td></td>
<td>0.13 %</td>
</tr>
<tr>
<td>Glycine Buffer</td>
<td>20 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>0.95 g/L</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vial R2a</th>
<th>ANTIBODY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse anti-human HbA1c monoclonal antibody</td>
<td>0.05 mg/mL</td>
</tr>
<tr>
<td>Buffer, Stabilizers</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vial R2b</th>
<th>ANTIBODY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat anti-mouse polyclonal antibody</td>
<td>0.08 mg/dL</td>
</tr>
<tr>
<td>Buffer, Stabilizers</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vial R3</th>
<th>HAEMOLYSIS REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous Solution Sodium Azide</td>
<td>0.5 g/L</td>
</tr>
</tbody>
</table>

**SAFETY CAUTIONS**

BILABO 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**

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.
**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**

<table>
<thead>
<tr>
<th></th>
<th>HbA1c</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGSP (%)</td>
<td>IFCC (mmol/mol Hb)</td>
</tr>
<tr>
<td>Non-diabetic:</td>
<td>&lt; 6.0 %</td>
<td>42</td>
</tr>
<tr>
<td>Glycemic control of a patient with diabetes:</td>
<td>&lt; 7.0 %</td>
<td>53</td>
</tr>
</tbody>
</table>

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.

- **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.
  - Hemolyzate Preparation: Lyse patient’s specimen, calibrators and controls as indicated in 5 Specimen Collection and Preparation.

**Calibration curve:**

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

**EXPECTED VALUES**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standards</th>
<th>Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex (Vial R1)</td>
<td>700 µL</td>
<td>700 µL</td>
<td>700 µL</td>
</tr>
<tr>
<td>Saline</td>
<td>20 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standards (4 different levels)</td>
<td>20 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen</td>
<td>20 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix well. Let stand for 5 minutes at 37°C.

**Anti-HbA1c(Reagent R2)**

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.

**CALCULATION**

Calculate the result as follows:

**Calculation:**

\[
\text{Abs} = \text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}
\]

**NGSP Results (%)**

Plot a Standard Curve \( y = \Delta \text{Abs} \).

Read the concentration of controls and patient specimens on the graph.

Results are reported as % HbA1c versus HbA.

**IFCC results (mmol/mol Hb):**

Use \( y = \Delta \text{Abs} \).

**IFCC (mmol/mol Hb) = \( \text{NGSP} \times 1.050 - 0.481 \times 10\% \**

**REFERENCES**

9. Recommandations HAS (antérieurement ANAES) relatives au “Suivi du patient diabétique de type 2 à l’exclusion du suivi des complications” (janv. 1999)