# Glycated Haemoglobin $A_1c$

**Chromatographic-spectrophotometric Ion exchange method**

**Code HB031 20 tests**

**Stored at 2-25°C.**

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## Clinical Significance

**Diabetes Mellitus is a chronic disease characterized by a hyperglycemia. The consequences are metabolism disorders of carbohydrates, lipids and proteins. The risk of complications associated with diabetes, including nephropathy, retinopathy and cardiovascular diseases, increases in patients with poor metabolic control. In the diabetic patients, where blood glucose levels are elevated, $HbA_1c$ is formed as a consequence of the non-enzymatic glycation of the N-terminus of the β-chain of haemoglobin molecule. The level of $HbA_1c$ is proportional to the level of glucose in the blood and has been widely accepted as an indicator of the mean daily blood glucose concentration over the preceding 6-8 weeks. It is therefore, a long-term indicator of diabetic control, whereas, the measurement of blood glucose is only a short-term indicator.**

## Principle

**After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Hemoglobin $A_1c$ is specifically eluted after washing away the $HbA_1a+b$ fraction, and is quantified by direct photometric reading at 415 nm.**

## Reagents

| | | |
|---|---|---|
| Reagent 1 (1x6 ml) | Potassium biphtalate Detergent, pH 5.0 | 50 mmol/l |
| Reagent 2 (1x 47 ml) | Phosphate buffer, pH 6.5 Sodium azide | 48 mmol/l 0.95 g/l |
| Reagent 3 (2x180 ml) | Phosphate buffer, pH 6.4 Sodium azide | 72 mmol/l 0.95 g/l |
| Reagent 4 20 pcs | Microcolumns: Contain a pre-weighted amount of resin equilibrated with phosphate buffer. | |

**Use only columns and reagents 2 or 3 of the same lot number. Because the reagents contain sodium azide, it is advisable not to pipet them by mouth.**

**For *in vitro* diagnostic use only.**

## Preparation

**Reagents are ready to use.**
**Bring the column and reagents to room temperature (21-26°C), a few minutes before use.**
**Column preparation: Always remove the upper cap from the column first and the lower cap second. Then, using the rounded end of a pipette, push the upper disc down to the resin surface taking care not to compress it. Let the column drain completely to waste.**

## Storage and stability

**All the components of the kit are stable at 2-25°C up to the date of expiration as specified.**

## Additional equipment

**- Spectrophotometer or colorimeter measuring at 415 nm**
**- Matched cuvettes 1,0 cm light path**
**- Test tubes (16x160 mm)**
**- General laboratory equipment**

## Samples

**Whole blood collected in heparin or EDTA. Stable for at least 7 days at 2-8°C**

## Procedure

1. **Wavelength 415 nm (405-425); Temperature 21-26°C; Cuvette 1 cm light path.**
2. **Adjust the instrument to zero with distilled water.**
3. **Hemolysate preparation and labile fraction elimination**

   **Pipette into a test tube:**

| | |
|---|---|
| Blood | 50 µl |
| Reagent 1 | 200 µl |

**Shake thoroughly and let it stand at room temperature for 10-15 minutes. The hemolysate will be used in steps 5 and 6.**

4. **Prepare a column: see preparation**
5. **Separation and reading of $HbA_1c$ fraction:**

   **Carefully pipette on the upper filter:**

| | | |
|---|---|---|
| Hemolysate | 50 µl | Let the column drain to waste |
| In order to drain any sample residue left above the upper disc, pipette: | | |
| Reagent 2 | 200 µl | Let the column drain to waste |
| Pipette: | | |
| Reagent 2 | 2.0 ml | Let the column drain to waste |
| Place the column over a test tube (16 x 100 mm) and add: | | |
| Reagent 3 | 4.0 ml | Collect the eluate ($HbA1c$ fraction) |
| Shake thoroughly and read the absorbance (Abs) of the $HbA_1c$ fraction at 415 nm against distilled water ($Abs_{HbA1c}$) | | |

6. **Reading of $Hb_{TOTAL}$**

   **Pipette into a test tube (16x160 mm):**

| | |
|---|---|
| Reagent 3 | 12.0 ml |
| Hemolysate | 50 µl |
| Shake thoroughly and read the absorbance (Abs) at 415 nm against distilled water ($Abs_{HbTOTAL}$) | |

## Calculation

**$\%HbA_1c$**
**$= Abs_{HbA1c} / (3 \times Abs_{HbTOTAL}) \times 100$**

## Reference values

| | |
|---|---|
| Normal range: | 4.2 – 6.2 % |
| Good controlled diabetic: | 6.7 – 9.1 % |
| Uncontrolled diabetic | > 9.1 % |

**This range is given for orientation purpose only. Each laboratory should establish its own reference range.**

## Performance characteristics

***Measuring range:*** **from 4.3% (detection limit) to at least 17.0%.**
***Precision:***

| | Intra-assay (n=25) | | Inter-assay (n=25) | |
|---|---|---|---|---|
| Mean (mg/dl) | 7,2 | 9,9 | 7,2 | 9,9 |
| CV (%) | 5,4 | 6,3 | 7,3 | 5,9 |

## Interferences

**Erroneous values might be obtained from samples with abnormally elevated quantities of other hemoglobins as a result of either their simultaneous elution with $HbA_1c$ (HbF) of differences in their glycation from that of HbA (HbS).**

## Notes

1. **The obtained values are temperature-independent when working in the recommended interval (21 – 26°C). If working temperature is out of range, multiply the obtained value by the corresponding factor showed in the following table:**

   | Working temp. | Factor |
   |---|---|
   | 18°C | 1.29 |
   | 19 °C | 1.18 |
   | 20°C | 1.12 |
   | 27-30°C | 0.91 |

2. **The long-term storage of the columns leads to an excessive packing of the resin diminishing the flow rate and lengthening the elution step. To regain the flow efficiency it is advisable - 10 min. before starting the test - to invert the columns to resuspend the contents, place them back to their upright position and let the resin settle for a few minutes**
3. **Some air bubbles may occasionally appear inside the resin bed. Their presence do not alter the test performance.**

## Bibliography


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