

Quantitative determination of HbA_{1c} in human blood
IVD (Microcuvettes)

Store 2 - 8°C.

PRINCIPLE OF THE METHOD

The HbA_{1c} is a quantitative turbidimetric test for the measurement of glycated hemoglobin in human whole blood. Total hemoglobin and HbA_{1c} have the same unspecific absorption rate to latex particles. When mouse antihuman HbA_{1c} monoclonal antibody is added (R2), latex-HbA_{1c}-mouse anti human monoclonal antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA_{1c} adsorbed onto the surface of latex particles. The amount of agglutination is measured as absorbance.

CLINICAL SIGNIFICANCE¹⁻³

Diabetes Mellitus is a chronic disease characterized by a hyperglycaemia. 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 ^{Note 1}

REAGENTS

Reagent 1	Latex particles 0.13%, Glycine buffer 20 mmol/L
Reagent R2A	Glycine buffer 80 mmol/L
Reagent R2B	Mouse anti-human Hba1c monoclonal antibody 0.05 mg/mL Goat anti-mouse IgG polyclonal antibody 0.08 mg/dL Stabilizers
Reagent 3	Hemolysis reagent

PREPARATION
Reagent 1 (R1): Ready to use.

Reagent 3(R3): Ready to use.

Reagent 2 (R2): Mix 1 part of R2B with 19 parts of R2A (1:20 dilution).

Samples and/or controls: To determine HbA_{1c}, a hemolysate must be prepared for each sample as follows:

- 1) Dispense 100 parts of hemolysis reagent (R3) in a tube
- 2) Add 1 part of well mixed whole blood and mix (1:101 dilution)
- 3) Allow to stand for 5 minutes or until complete lysis is evident.

STORAGE AND STABILITY

 Whole blood collected with EDTA: 7 days at 2-8°C
 90 days at -15°C

 Hemolysate: 10 days at 2-8 °C
 R1, R2A, R2B and R3: up to the expiration date at 2-8°C
 R2 (R2A+R2B): 30 days at 2-8°C

Reagent deterioration: Protect reagents from extreme heat and light. Do not freeze.

ADDITIONAL EQUIPMENT

- Pipette 100 - 1000 µL
- Pipette 10 - 100 µL
- **Microcuvettes and stirrers**

N.B.: This test requires the use of MICROCUVETTES (Ref.: ACC16-047)!
SAMPLES

Venipuncture or capillary blood samples may be used.

EDTA is recommended as anticoagulant.

Frozen samples should be thawed at room temperature, mixed thoroughly prior to use and should not be refrozen.

TEST PARAMETER

 Filter: set filter on position A
 Reading time: 300 seconds

PROCEDURE

1. Pipette into a Microcuvette:

kind of reagent	µL
R1	300
Hemolysate	25

2. Add a stirrer to each microcuvette and incubate microcuvettes in position 1-4 for exactly 5 minutes
3. Transfer each microcuvette in the reading channel and, when requested on the display, add 100 µL of R2
4. Read the result which will appear automatically on the reader's display after 300 sec.

QUALITY CONTROL

HbA_{1c} Direct Control (ref.: ACC16-050) is recommended to monitor the performance of the assay procedure. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES
According to NGSP:
Non-diabetics: <6%.

Controlled diabetics: <7%.

Each laboratory should establish its own reference range to reflect the age, sex, diet and geographical location of the population.

PERFORMANCE CHARACTERISTICS

1. *Assay range:* 0 to 15%, under the described assay conditions. Samples with values above >= 15% HbA_{1c} should be diluted with saline solution and re-tested.

2. *Precision:*

	Intra-assay (n=20)	Inter-assay (n=20)
Mean %	13.1	12.3
SD	0.8	1.1
CV (%)	6.1	8.9

3. *Correlation:* Results obtained using this reagent (y) were compared to those obtained using a NGSP certified procedure (x). 80 samples were assayed. The correlation coefficient (r) was 0.989 and the regression equation $y = 1.032x - 0.040$.

INTERFERENCES⁴⁻⁸

No interferences up to:

Bilirubin 50 mg/dL, tryglicerides 2000 mg/dL, ascorbic acid 50 mg/dL, carbamylated Hb 7.5 mmol/L, acetylated Hb 5.0 mmol/L.

It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead poisoning, alcoholism, ingest large dose of aspirin. Elevated levels of HbF may lead to underestimation of HbA_{1c} and that Uremia does not interfere with this test. HbS and HbA₂ are not detected by immunoassay, leading to possible inaccurate determination. Also it has been reported that labile intermediates like Schiff Base are not detected and do not interfere with HbA_{1c} determination.

NOTES

1. Clinical diagnosis should not be made on a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Wolf HU et al. Clin Chem Acta 1984; 136: 83-104.
2. Nathan DM et al. Mne J Med 1094; 310: 341-346.
3. Goldstain DE et al. Clin Chem 1986; 32: B64-B70.
4. Ceriello A. et al., Diabetologia 22, p. 379 (1982)
5. Little R.R. et al., Clin. Chem. 29, 99 466-469 (1983)
6. Fluckiger R. et al., New Eng. J. Med. 304, pp. 823-827 (1981)
7. Nathan D.M. et al., Clin. Chem. 35, pp. 466-469 (1983)
8. Engbaek F et al., Clin. Chem. 35, pp 93-97 (1989)

PACKAGING – REF: ACC16-048

R1	1 x 30 mL
R2A	1 x 9.5 mL
R2B	1 x 0.5 mL
R3	1 x 125 mL