CHRONOLAB

CE

IVD

Glycated Hemoglobin

Latex turbidimetry

Quantitative determination of glycated hemoglobin ($HbAB_{1BC}$)

PACKAGING

Ref.: 101-0557	Cont.: 80 tests
Ref.: 101-0558	Cont.: 200 tests

Store 2 - 8º C.

CLINICAL SIGNIFICANCE [1-3]

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, HbA1c is formed as a consequence of the non-enzymatic glycation of the N-terminus of the β-chain of haemoglobin molecule. The level of HbA1c 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 [1].

PRINCIPLE OF THE METHOD

The HbA1c is a quantitative turbidimetric test for the measurement of glycated hemoglobin in human whole blood. Total haemoglobin (HbT) and HbA1c concentrations are measured and the HbA1c result is calculated as a percentage of the HbT concentration.

After lysis of red blood cells, the haemoglobin molecule is hydrolyzed by a protease and haemoglobin derivates are converted to alkaline hematin, which is measured by absorption at 600 nm. HbA1c is then measured using a latex agglutination inhibition rate assay. Latex particles coated with specific monoclonal anti- human HbA1c compete for agglutination with HbA1c of the sample when mixed with a second reagent constituted by polymer particles coated with HbA1c. The presence of HbA1c in the sample inhibits the agglutination of latex particles. The agglutination grade is indirectly proportional of the HbA1c concentration in the sample, and can be quantified by comparison from a calibrator of known HbA1c concentration.

REAGENTS

Haemolyzing Reagent (R3)	Porcine pepsin in buffer solution. Preservatives. pH, 2.4
Total Hb Reagent (R4)	Sodium hydroxide 0.4%, Triton 2.5%. pH, 13
HbA1c– Latex (R1)	Anti-HbA1c monoclonal antibody (mouse) coated to latex particles, BSA, non-ionic surfactant 0.6% and Proclin 150 0.1%, in a buffer pH, 8.1.
Agglutinator Reagent (R2)	HbA1c hapten covalently attached to a polymer, BSA, non-ionic surfactant 0.2% and Proclin 150 0.1%. pH, 2.0.
Optional	Ref: 101-0559 HbA1c Calibrator Set Ref: 101-0560 HbA1c Control Set

PRECAUTIONS

Total Hb Reagent contains sodium hydroxide, which is caustic, causes severe burns. Use gloves. In the event of accident, flush the effected area with plenty of water and seek immediate medical attention.

CALIBRATIONP [7-9]

The sensitivity of the assay and the target value of the calibrator have been standardized against an Internal Reference Material HbA1c traceable to the HPLC method for HbA1c and Drabkin's method for Total Hb. It is not recommended the use of other commercially available HbA1c calibrators.

Total Hb test and HbA1c test: Chronolab recommends using HbA1c Calibrator (Ref: 101-0559).

Calibrators do not require pretreatment.

PREPARATION

Reagents: Ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C and contaminations are prevented during their use. Do not use reagents over the expiration date.

Reagent deterioration: Presence of particles and turbidity. Protect reagents from extreme heat, light or freezing.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37º C.
- Spectrophotometer or photometer thermostatable at 37º C with a 600 nm (580 – 620) filter.

SAMPLES

Venipuncture or capillary blood samples may be used.

Potassium-EDTA or Ammonium Heparin are recommended as anticoagulants.

Stable 7 days at 2-8º C or 3 months at –20º C or 3 days at 15-15º C.

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

SAMPLE TREATMEENT

1. Prepare in a tube a 1/41 dilution of the sample:

   10 µL whole blood sample + 400 µL Haemolyzing Reagent (R3)

2. Seal the tube and mix avoiding foaming.
3. Incubate for a minimum of 5 minutes at room temperature.

Stability: Treated sample is stable up 2 hours at room temperature or 8 h at 2-8º C.

PROCEDURE

Total Hb test

1. Bring the Total Hb Reagent (R4) and the photometer (cuvette holder) to 37º C.
2. Assay conditions:
   Wavelength: 600 nm (580-620)
   Temperature: 37º C
   Cuvette ligth path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Total Hb Reagent (R4) (mL)	1.0
Calibrator (0 and 1)[2] or sample (µL)	80

5. Mix and read the absorbance after 5 minutes (A) of the sample addition.

HbA1c test

1. Bring the HbA1c-Latex (R1) and Agglutinator (R2) Reagents and the photometer (cuvette holder) to 37º C.
2. Assay conditions:
   Wavelength: 600 nm (580-620)
   Temperature: 37º C
   Cuvette ligth path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

HbA1c-Latex Reagent (R1) (µL)	500
Calibrator (0 to 6) or sample (µL)	20

5. Mix and incubate 5 minutes.
6. Pippete into the cuvette:

Agglutinator Reagent (R2) (µL)	475

7. Mix and read the absorbance immediately (AB_{1B}) and after 2 minutes (AB_{2B}) of the R2 addition.

Chronolab has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

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CALCULATIONS

Total Hb concentration (g/dL)

Plot the (A) obtained against the THb concentration of each calibrator (0 and 1 Level). Total Hb concentration in the sample is calculated by interpolation of its (A) in the calibration curve.

HbA1c concentration (g/dL)

Plot ($AB_{2\ B}$– AB_{1B}) obtained against the HbA1c concentration of each calibrator (1 to 6 Level). HbA1c concentration in the sample is calculated by interpolation of its (AB_{2B}- AB_{1B}) in the calibration curve.

U**% HbA1c:**

The calculation of the HbA1c concentration is generated using the following equation:

$$\%HbA_1c = \frac{HbA_1c\ (g/dL)}{T.Hb\ (g/dL)} \times 100$$

QUALITY CONTROL

HbA1c Control (ref: 101-0560) is recommended to monitor the performance of manual and automated assay procedures. **Controls require pretreatment after being reconstituted.**

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Non-diabetics: Between 4-6 %.
Controlled diabetics: Between 6-8 %.
Uncontrolled diabetics: >8 %.

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

PERFORMANCE CHARACTERISTICS

1. **Linearity limit (Total Hb)**: 7 to 23 g/dL, under the described assay conditions.
2. **Assay range (HbA1c):** Approximately 0.3 to 2.06 g/dL, under the described assay conditions. These values are dependent on the lot specific values of the calibrators in use.

   % HbA1c = 14.7% i.e.⇒ 2.06 g/dL in 14 g/dL T Hb.

   Samples with values above 14.7% HbA1c should be diluted and results should be reported as > 14.7% HbA1c.

3. **Detection limit:**
   HbA1c: Values less than 0.3 g/dL, give non-reproducible results.
   T Hb: 1.38 g/dL, give non-reproducible results.

   In practice, the sensitivity of T Hb is 7 g/dL as disease states that result in a lower concentration of T Hb will result in a change of HbA1c level, giving an inaccurate result.

   % HbA1c = 2.57 % i.e. ⇒ 0.3 g/dL HbA1c in 11.667 g/dL T Hb.

4. **Precision:**

	Intra-assay (n=20)			Inter-assay (n=20)		
Mean (g/dL)	5.51	8.28	10.7	5.56	8.49	10.9
SD	0.001	0.006	0.004	0.002	0.003	0.005
CV (%)	2.5	6.67	4.0	3.77	3.63	4.82

6. **Correlation:** Results obtained using this reagent (y) were compared to those obtained using another commercially available method (x) with similar characteristics. 40 samples ranging from 5.4 to 12.3 % of HbA1c were assayed. The correlation coefficient (r) was 0.9747 and the regression equation y = 0.96x +0.4532.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES[10]

Bilirrubin (30 mg/dL), tryglicerides (1.6 g/dL) and rheumatoid factors (2000 IU/mL), acetylsalicylic ac. (60 mg/dL), sodium cyanate (50 mg/dL) and urea (500 mg/dL) do not interfere. The labile fraction of glycated hemoglobine does not interfere, as the antibody is specific for the stable ketamine. Other substances may interfere.

NOTES

1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
2. Use NaCl 9g/L as a Calibrator 0.

BIBLIOGRAPHY

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