HbA1c (LETIA)

INTENDED USE

Bioline HbA1c is used for the quantitative determination of HbA1c in whole blood.

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, HbA1c is formed as a consequence of the non-enzymatic - chain of haemoglobin molecule.βglycation of the N-terminus of the 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

METHOD AND PRINCIPLE

This reagent provides a quantitative assay for measuring concentrations of HbA1c in whole blood or red blood cell. It is based on latex agglutination. HbA1c test samples are absorbed onto the surface of latex particles, which react with Anti-HbA1c (antigen-antibody reaction). The turbidity caused by latex agglutination is measured at 660 nm, and the HbA1c concentration in whole blood or red blood cell is calculated from calibration curve.

REAGENT COMPOSITION

R1; Latex solution

R2; Mouse anti-HbA1c monoclonal antibody, Goat Anti-mouse IgG

Calibrator - Lyophilized . Store at 2 - 8 ºC.

Check the calibrator concentration on the bottle label. After reconstitution, the calibrator is stable for 7 days at 2 - 8 ºC and 30 days at -20 ºC.

The calibration is valid only for the reagents and calibrator from the same lot number. **Reagents and calibrators are not interchangeable between kits with different lot numbers**.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use.

To be handled by entitled and professionally educated person Reagents of the kit are not classified like dangerous but contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

2. Reagents S25: Avoid contact with eyes.

3. In very rare cases, samples of patients with gammopathy might give falsified results.

4. Immediately after HbA1c measurement cleaning of cuvettes is necessary. Use the alkaline cuvette washing solution which is recommended by the analyzer manufacturer.

5. Take necessary precautions for use of laboratory reagent.

REAGENT PREPARATION

Reagent R1 and R2 are ready to use as supplied. Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label.

REAGENT DETERIORATION

1. Avoid R-1 Latex from freezing or drying because it may cause non-specific agglutination. Discard the reagent if it does not meet stated performance parameters.

SPECIMEN COLLECTION AND LYSING.

Whole blood collected with EDTA. Lysing procedure below

Distilled water	500 µl
Whole blood Sample	10 µl

Mix and allow to stand for 5 minutes or until complete lysis is apparent.

MANUAL ASSAY PROCEDURE

1st reaction

Add 375 µL of R-1 Latex to 15 µL of the hemolyzed test sample or each HbA1c calibrator, and after mixing the solution, incubate it for five minutes at 37°C.

2nd reaction

Add 125 µL of R-2 solution and after mixing, incubate for five minutes at 37 °C.

Measure absorbance (turbidity) of each test sample and respective HbA1c calibrators (at 660nm) according to the operating procedures for the autoanalyzer.

Calculation of HbA1c concentration:

According to the operating procedures for the autoanalyzer, calculate the concentration of HbA1c in the test sample.

When concentration (%) of test samples exceed the measurable range, dilute the test samples by 2-3 times with HbA1c calibrator STD-2, or another test sample with a known concentration (%) of HbA1c. Then run the test again and calculate the concentration (%) of HbA1c from the following calculation formula;

Concentration(%) of HbA1c

= [retested HbA1c (%) x n] - [HbA1c (%) of the sample/ a calibrator used for the dilution x (n-1)]

n = Dilution factor of the test sample (2-3).

[Calculation example for a test sample which exceeds the measurable range]

When adding 200 ?L of a known sample (HbA1c 4.5%) to 100 ?L of a test sample with approximately 17%, obtained by using the test on the undiluted test sample. This means three times dilution. If at retest, 9.1 % was obtained, the correct concentration (%) of the test sample is calculated by following the calculation formula;

Concentration (%) of HbA1c = (9.1 x3)-(4.5x(3 - 1)) = 27.3 - 9.0 = 18.3 %

LIMITATIONS

Due to its antibodies, HbA1c is a specific immunoassay for human HbA1c. No interference was observed by ascorbic acid up to 60 mg/dl, conjugated and unconjugated bilirubin up to 40 mg/dl, lipemia up to 2000 mg/dl triglycerides, RF up to 250 IU/ml, carbamylated Hb up to 7.5 mmol/l, and acetylated Hb up to 5.0 mmol/l.

No interference is observed by uremia, labile intermediates (Schiff base), and Hemoglobin variants HbS and HbA2. Elevated levels of HbF may lead to falsely low HbA1c values. Alcoholism and ingestion of large doses of aspirin may lead to inconsistent results.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with the established Hba1c values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

4.6-6.2% (NGSP)

Reference intervals should be established or verified by the laboratory based on an appropriate non-diabetic patient population.

	%NGSP	%IFCC	mmol/mol
Non Diabetic	4-6	3-4	30-40
Target of Therapy	<7	<5	<50
Change of therapy	>8	>6	>60

PERFORMANCE CHARACTERISTICS.

Measuring range: 3.3 to 16.0 %

Specificity: When 2 HbA1c control samples with a known concentration (%) (high, middle, low) are tested according to the test procedure described in the package insert, each test result should be within ± 20% of respective known concentrations (%) of the HbA1c control sample.

Sensitivity: When absorbance of HbA1c calibrator STD-1(0%) is measured according to the test procedure described in the package insert, the absorbance (turbidity) at 660 nm should be 0.70 or less.

(2) When absorbance of HbA1c calibrator STD-5(15.6%) is measured according to the test procedure described in the package insert, the absorbance (turbidity) at 660 nm should be 1.00 or more.

Method Comparison:

Study results in comparison with HPLC method.

n = 81

Correlation coefficient: r = 0.997

Regression equation: y = 0.979x + 0.0252

General Technical Parameters

Mode	**End Point**
Wavelength (Filter)	**660 nm**
Reaction Direction	**Increasing**
Reagent Blank	**Yes**
Lysate Sample Vol.	**15 µL**
Reagent Vol.	**500 µL**
Incubation Time	**5+5 min**
Reagent Blank Abs.(Max)	**NMT 0.700 Abs**
Calibration Method	**Multipoint**
Standard (Conc.)	**Refer calibrator vials**
Linearity	**16%**
Decimal Places	**1**
Temp.	**37°C**
Unit	**%**
Ref. Low (Male / Female)	**4.6%**
Ref. High (Male / Female)	**6.2%**

REFERENCES

1.Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 142-48.

2. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999.p. 790-6.

3. Jeppsson JO, Kobold U, Barr J, Finke A et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002;40:78-89.

4. Hoelzel W, Weykamp C et al. IFCC Reference System for Measurement of Hemoglobin A1c in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study. Clin Chem 2004; 50:1:166-74.