BM Test HbA1c

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General Precautions

1. **BM Test HbA1c** is for in vitro diagnostic use only.
2. Clinical diagnosis should not be based on the results of this product only; other test results and clinical symptoms should also be taken into consideration.
3. Follow the instructions in the package insert for proper use. The reliability of the test results cannot be guaranteed if the proper procedure is not followed or if the kit is used for purposes other than those specified in the package insert.
4. In case of contact with mouth or eyes, wash the contact area with plenty of water and seek medical advice if necessary.
5. Read the instructions for use of your clinical chemistry analyzer carefully before using the kit. Test parameters for various BioMajesty analyzers are available from JEOL Ltd.
6. Perform quality control and ensure the accuracy on your clinical chemistry analyzer before using the kit.

Kit Composition

Reagent 1; 10-(carboxymethylaminocarbonyl)-3,7-bis (dimethylamino) phenothiazine, sodium salt 10 – 30 nmol/mL

Reagent 2; Peroxidase (POD) 50 – 150 U/mL
Fructosyl peptide oxidase (FPOX) 3 – 9 U/mL

Summary

This kit is for the quantitative measurement of the percentage of glycated hemoglobin levels in human whole blood.
HbA1c is a glycated product of hemoglobin A_0 that is a major component of hemoglobin. The level of the HbA1c reflects the mean value of blood glucose concentration over the previous few weeks. Therefore, it is considered to be a crucial diagnostic test for controlling the blood glucose levels.

Principle

1. Assay principle

In the first reaction, the hemoglobin concentration is simultaneously measured at 478 nm/805 nm as fructosyl dipeptides are generated from the N-terminus amino groups of the beta-chain of HbA1c by the reaction of protease.
In the second reaction, the fructosyl dipeptides react with fructosyl peptide oxidase, generating hydroperoxide. The hydroperoxide allows 10-(carboxymethylaminocarbonyl)-3 and 7-bis (dimethylamino) phenothiazine sodium salt to develop a color in the presence of peroxidase. The change in absorbance at 658 nm/805 nm is measured for HbA1c concentration.
The percentage of HbA1c in the total hemoglobin is calculated using both results of hemoglobin concentration and HbA1c concentration.

2. Characteristics
   1) **BM Test HbA1c** is an enzymatic reagent specific to N-terminal fructosyl dipeptides of the beta-chain.
   2) **BM Test HbA1c** is a ready-to-use liquid reagent.
   3) **BM Test HbA1c** does not contaminate to the reaction cuvettes after use.
   4) **BM Test HbA1c** is not susceptible to the influence of labile HbA1c and modified hemoglobin (carbamylated or acetylated hemoglobin).

Sample Preparation

1) Use venous blood in NaF or EDTA or Heparin tube and use **BM Test HbA1c Diluent** (JEOL Ltd.) for hemolysis. Please note that grossly hemolyzed blood must NOT be used.
2) Venous blood samples (not pretreated by **BM Test HbA1c Diluent**) are stable for 7 days when refrigerated.
3) Venous blood shall be centrifuged (800g, 5 minutes) before measurement.

Precautions

1. Interference substances
   1) The following substances do not affect the measurement results:

      Free bilirubin: up to 50 mg/dL
      Conjugate bilirubin: up to 50 mg/dL
      Ascorbic acid: up to 50 mg/dL
      Intralipos: up to 2%
      Formazin turbidity: up to 3000 units.
      Hemoglobin modified by acetaldehyde, acetylsalicylic acid or sodium cyanide that is added to the sample (acetaldehyde-Hb, acetylated Hb or carbamylated Hb): up to 50mg/dL
      Labile HbA1c : up to 1000mg/dL
      Hb variants (HbD, HbE, HbC, HbS) do not affect the measurement results. [4]
   2) Take extra care of the samples from patients who are prescribed amino-acid transfusion. Avoid using samples of those patients who have been given a transfusion with glucose and amino acid as these samples may show falsely high results.
   3) Interference reactions with substances other than those intended may occur. If a measurement value or test result does not seem to be correct, retest other specimen or diluted samples, or confirm the test result using another method.
2. Others
   1) Use **BM Test HbA1c Calibrator** (JEOL Ltd.) for calibration. On-board calibration stability is 30 days.
   2) Dilute the venous blood sample with a twofold amount of **BM Test HbA1c Diluent** or redilute the pretreated sample and retest if hemoglobin concentration of the pretreated sample exceeds 310 µmol/L.
   3) Dilute increased amount of the venous blood sample with **BM Test HbA1c Diluent** and retest if the hemoglobin concentration is 90 umol/L or less.
   4) Tubes with gel separator or granulate cannot be used.
   5) Hb concentration observed from the measurement should not be used for diagnosis purpose.

Preparation and Procedure

1. Preparation of reagents
   1) Reagent 1: Ready-to-use
   2) Reagent 2: Ready-to-use

2. Procedure

   The following procedure is for use with a BM 6010.
   The sample is taken from the blood cells in the lower part of the tube and pretreated with **BM Test HbA1c Diluent**.

   Reagent 1 90 µL
   Pretreated sample 12 µL
   Mix, read absorbance for Hb measurement, then add
   Reagent 2 30 µL

Mix, read absorbance for HbA1c measurement.
Difference in absorbance at 478 nm and 805 nm for Hb measurement and at 658 nm and 805 nm for HbA1c measurement.

Reference Range [1]

Cut-off point for the diagnosis of diabetes:
>= 6.5 % (NGSP) (47.5 mmol/mol (IFCC))

Performance

1. Sensitivity
   1) Hb; 0.10 - 0.30 abs. per 100 µmol/L Hemoglobin
   2) HbA1c; 0.050 - 0.100 abs. per 10 µmol/L HbA1c
2. Accuracy
   Within 90 – 110 % to the expected assay values
3. Precision
   Coefficient of variation of =< 5 % (within-run)

A.m. results are based on the in-house test procedures.

4. Measuring Range (for BM6010)
   3.0 - 16.0 %: NGSP (9.3 – 151.4 mmol/mol: IFCC)
   HbA1c (in the case of Hb concentration ranging from 90 to 310 µmol/L)
5. Correlation
   N = 68
   Correlation coefficient: r = 0.989
   Regression equation: y = 0.99 x - 0.12
   Comparative method: HPLC method
   y: **BM Test HbA1c** x : Comparative method
6. Standard material;
   JCCRM 411

Standardization [2][3]

The assay is standardized according to the IFCC reference method. Calibration according to NGSP is possible. Corresponding calibrator values are listed on the label of the calibrator.
IFCC and NGSP values show a liner relationship and can be calculated from each other using the following equation.

NGSP (%) = IFCC (mmol/mol) x 0.0915 + 2.15

Precautions and Warnings

1. Precautions for handling (Hazard prevention)
   1) Handle all samples used in the test as potentially HIV, HBV or HCV infective. To prevent the risk of infection, use disposable gloves and avoid pipetting by mouth.
   2) Reagent 1 contains Proclin 300, which may cause skin-irritation, as a preservative. If the reagents come into contact with skin or clothes, rinse immediately with plenty of water and consult a doctor if skin irritation persists.
   3) Reagent 1 contains sodium azide. If the reagents come into contact with eyes, mouth or skin, rinse immediately with plenty of water as first aid and consult a doctor if required.
   4) Reagent 1 contains N,N-dimethylformamide. If the reagents come into contact with eyes, mouth, or skin, rinse immediately with plenty of water as first aid and consult a doctor if necessary.
2. Precautions for Use
   1) Store this product tightly sealed and avoid freezing. Do not use the reagents that are once frozen or opened as they can be deteriorated and may give inaccurate results.
   2) Do not use the reagents after the expiration date printed on the label. Reliability of the measurement values cannot be guaranteed if such reagents are used.
   3) Do not replenish the reagent.
   4) Avoid direct sunlight in the work area when running assays.
   5) As there is a risk of assay results being artificially high in a sub-portion of samples with extremely low peroxidase (POD)-like activity, physicians should assess all results comprehensively in conjunction with clinical symptoms and other findings.
3. Precautions for Disposal
   1) Before disposal, the remaining samples and accessories must be soaked in sodium hypochlorite solution of a concentration higher than 0.1 % for more than an hour or autoclaved at 120 °C for 20 min.
   2) If a sample or hemolyzed sample is accidentally splashed, wipe the affected area thoroughly with sodium hypochlorite solution of a concentration higher than 0.1 % to avoid possible infection.
   3) Before disposal, the reagents, samples processed with reagents and accessories should be separated into different types of wastes, such as medical wastes and industrial wastes, in accordance with the waste disposal regulations.
   4) Dispose the reagents and containers in accordance with the local environmental regulations related to water pollution control.
   5) Reagent 1 contains sodium azide. It can react with lead or copper pipes to produce the highly explosive metal azide. Ensure to flush the reagent with large volumes of water when disposing the product.
4. Other Precautions
   Do not use the bottles and caps for other purposes.

Storage instructions and Reagent stability

The reagents are stable for 12 months after production date, if stored at 2 – 10 °C, and R1 shall be protected from light.

Package

Reagent 1	2 x 30mL
Reagent 2	2 x 10mL

References

1. International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes: Diabetes Care, 2009 ; 32:1327-1334
2. Jeppsson JO, et al; International Federation of Clinical Chemistry and Laboratory Medicine: Approved IFCC reference method for the measurement of HbA1c in human blood. Clin. Chem. Lab Med 40: 78-89, 2002
3. Cas Weykamp, et al. Clin Chem 54:240-248, 2008
4. In-house test data of JEOL Ltd.

Manufacturer

JEOL Ltd.
2-11-1 Inadaira, Musashimurayama, Tokyo
208-0023 JAPAN