HbA1c Reagent Kit Hb-20

Instructions for Use

Ref.:**6001**

Intended use . Reagents applied in the quantitative determination of Hemoglobin A1c (HbA1c) in whole blood samples using the high-performance liquid chromatography (HPLC) method with ion-exchange.

Professional use.

[For in vitro diagnostic use.]

Test principle . The principle of the method is based on the high-performance chromatographic separation of HbA1c using an ion-exchange column as the stationary phase. Non-HbA1c has a positive charge, while HbA1c is practically neutral. HbA1c can be separated from non-HbA1c due to the difference in their charges, as shown in Figure 1. The stationary phase has a group with exchangeable cations, which can bind to the positively charged non-HbA1c by electrostatic action. As HbA1c is uncharged, it cannot bind to the stationary phase. Therefore, elution is performed with a low ionic strength washing solution so that HbA1c is eluted first; subsequently, non-HbA1c is eluted with a high ionic strength washing solution to obtain the corresponding hemoglobin chromatogram.

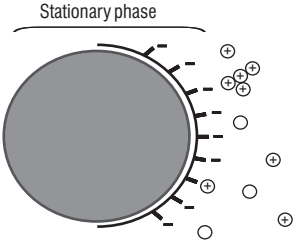

Figure 1 - Schematic diagram of the HbA1c separation principle.

Summary . HbA1c is widely used in clinical practice as a test indicator for diabetes. Because it is a product of the irreversible enzymatic reaction between human hemoglobin and blood glucose, HbA1c, it can reflect the average level of glucose in human blood within 2 to 3 months, and the test results are not interfered with by food.

Methodology . High Performance Liquid Chromatography (HPLC)

Reagents

Ready for use – Store between 4 - 30 ºC. Do not freeze.

R A **- HbA1c Reagent A**
Buffer ≤ 0,5%; Conservative≤ 0,05% and Sodium azide ≤ 0,02%.

R B **- HbA1c Reagent B**
Buffer ≤ 0,6%; Conservative ≤ 0,05% and Sodium azide ≤ 0,02%.

R C **- HbA1c Reagent C**
Buffer ≤ 0,5%; Conservative ≤ 0,05% and Sodium azide ≤ 0,02%.

R D **- HbA1c Reagent D**
Buffer ≤ 0,6%; Conservative ≤ 0,05% and Sodium azide ≤ 0,02%.

Stability . The reagents remain stable until the expiration date indicated on the label, as long as they are closed and stored away from light, at a temperature between 4-30 ºC.

Once opened, the reagents remain stable for 4 months when stored at 4-30 ºC.

Precautions and warnings

- Carefully read the label and instructions for use of other products relevant to carrying out the test (HbA1c Reagent H Vercentra Hb-20 and HbA1c Column) before use;
- Do not mix different batches of reagents, hemolyzer and columns;
- In case of skin and eyes contact, immediately flush eyes and skin with plenty of water and get medical assistance;
- In case of accidental ingestion, get medical assistance;
- Disposal of all waste material should be in accordance with local guidelines.

Applicable instrument . The HbA1c Reagent Kit Hb-20 product is suitable for the Vercentra HB-20 analyzer.

Material required not provided

1. Vercentra HB-20 Analyzer
2. HbA1c Reagent H Vercentra Hb-20
3. Calibra Vercentra HbA1c
4. Qualitrol Vercentra HbA1c
5. HbA1c Column

Sample

A Standard Operating Procedure (SOP) must be created to establish adequate procedures for sample collection, preparation and storage. The pre-analytical errors can be more demaging than the ones which may occur during the analytical procedure.

Use venous blood samples collected using a vacuum blood collection tube containing the EDTA-K2 anticoagulant. Blood samples should not be stored for more than 5 days at 2-8 ºC or should not be stored for more than 30 days at -20 ºC.

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Sample preparation

1. It is not necessary to prepare the sample. The blood collection tube can be placed directly into the equipment.
2. If the volume of the whole blood sample in the blood collection tube is less than 1 mL, homogenize the sample well and subsequently perform a pre-dilution in a proportion of 1:250 with the hemolyzer (HbA1c Reagent H Vercentra Hb-20). To perform the test, the pre-diluted samples must be placed in the sample cuvettes.

Interference

Bilirubin concentrations up to 18 mg/dL, hemolytic hemoglobin up to 450 mg/dL, chyle up to 1,400 FTU, ascorbic acid up to 50 mg/dL, glucose up to 1,200 mg/dL and acetaldehyde up to 60 mg/dL do not produce significant interferences.

Samples from patients with hemolytic anemia may show a reduced HbA1c value due to the shorter lifespan of their red blood cells. The magnitude of this effect depends on the severity of the anemia. Samples from patients with polycythemia or post-splenectomy may present an increased HbA1c value due to the relatively long lifespan of their red blood cells.

General instructions for use

1. Open the HbA1c Reagent Kit Hb -20 and remove one vial of each elution reagent: HbA1c Reagent A, HbA1c Reagent B, HbA1c Reagent C and HbA1c Reagent D. Place them in the corresponding positions of the Vercentra HB-20 analyzer and connect the reagent bottles to reagent lines according to labels. Tighten the caps on the reagent bottles. After installing or replacing the elution reagent(s), touch the "Perfusion" and "Rinse " buttons on the menu of the Vercentra HB-20 analyzer;
2. Place the sample rack carrying the whole blood or diluted samples into the sample rack tray. Tap the "Analysis" button on the main interface, and then tap the "Run" button after entering the analysis interface. The instrument will automatically recognize the sample mode and start the test. The sample will automatically diluted and injected into the chromatography column. According to the pre-set procedure of the instrument, first inject the low concentration HbA1c Reagent A, then inject the HbA1c Reagent B and HbA1c Reagent C, and finally inject the high concentration HbA1c Reagent D. Each hemoglobin is detected successively by the detector at 415nm. HbA1c% = HbA1c peak area / total hemoglobin area. The test results will be displayed on the analysis interface, and the test results can be printed directly.
3. To make the results more accurate, the system must be calibrated as follows:
   a) Take out the calibrator and warm it to room temperature. Add the specified amount of HbA1c Reagent H Vercentra Hb-20 to the calibrators. Wait until all the material is solubilized. Prepare the calibrators according to your specifications.
   b) Transfer the solubilized calibrators to the sample cups and place them in the first and second positions, respectively. Then place the sample rack on the sample rack tray.
   c) Tap the "Calibration " button on the main interface of the analyzer to go to the calibration interface and set the calibration as instructed by the Vercentra HB-20 analyzer. Tap the "Run" button after setting and the analyzer will automatically start the calibration operation.
   d) After the calibration is completed, the instrument will automatically calculate the slope "F" and the intercept "O" of the calibration result.
4. Column: HbA1c Column 800T Ref. 6006-800, HbA1c Column 1600T Ref. 6007-1600 and HbA1c Column 2400T Ref. 6008-2400.
5. Calibrator: It is recommended to use Calibra Vercentra HbA1c Ref. 6004-2/5.

Calibration . The HbA1c concentration in the Calibra Vercentra HbA1c - Ref. 6004 is traceable to the internationally approved IFCC calibrator (HBA-IFCC-cal). Calibrators must be reconstituted and stored in accordance with the Calibra Vercentra HbA1c Instruction for Use - Ref. 6004

Internal quality control . The laboratory must keep an internal quality control program with well-defined regulations, objectives, procedures, criteria of quality specifications and tolerance limits, corrective actions and registration of activities. It is recommended to use the Qualitrol Vercentra HbA1c product for internal quality control of the system to determine the percentage of HbA1c.

Reference values . As recommended by the American Diabetes Association :
Non-diabetic Level: 4.0% ~ 6.0%
Criteria for diagnosing Pre-diabetic level: 5.7% - 6.4%
Criteria for diagnosis of diabetes level: ≥ 6,5% (threshold)
It is recommended that each laboratory establish its own reference values based on the characteristics of the population.

Interpretation of test results

1. The Vercentra HB-20 analyzer can automatically recognize the peak of each hemoglobin. The test results are directly displayed on the screen and no futher calculations are required.
2. Reportable range: The reportable range of the HbA1c assay kit is 3% to 20%. When the test result exceeds this range, the HbA1c test result is not reliable.
3. If samples are not stored as required by the manufacturer, these samples may be subject to change and results may be unreliable and should not be reported. It is recommended to repeat the test using fresh samples.
4. When HbA1c is correctly identified and shaded, it means that HbA1c has good separation, being calculated correctly by the software, and the result is reliable. Otherwise, you will need to verify that the reagents are installed correctly and that the instrument components are functioning properly.

Performance characteristics

1. Appearance and character: The components of the kit should be complete, neat in appearance, and clearly marked. The HbA1c Reagent A, HbA1c Reagent B, HbA1c Reagent C and HbA1c Reagent D of the HbA1c Reagent Kit Hb -20 Ref. 6001 are composed of colorless and transparent liquids, without precipitates.
2. Intra-run precision: A sample with a percentage between 4.0%~6.5% of HbA1c was determined 20 times using the same batch of product. A Coefficient of variation (CV) was obtained ≤ 2,0%.

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3. Inter-batch relative range: A sample with a percentage between 4.0%～6.5% of HbA1c was determined 3 times using three batches of the product. The relative range of results was ≤6.0%.
4. Accuracy: Two reference samples were determined 3 times using three batches of the product. The relative deviation between the target value and the found value was ≤6,0%.
5. Linear reange: A sample with a high percentage of HbA1c, close to the upper limit and a sample with a low percentage of HbA1c close to the lower limit of the operational range were diluted and determined in triplicate. The linear regression equation was determined finding a linearity of 3.0% to 20.0% and correlation coefficient (r) ≥ 0,9900.
6. Reagent volume: The product volume in each specification should not be less than the volume specified on the label.
7. Interferences: Hemoglobin variants can be detected by the equipment. Therefore, HbA1c results should not be affected by the presence of HbC, HbD, HbE and HbS in the heterozygous state.

Column information

Main components
Column: hydrophilic polymer, assembled column and sealing plug.
Accessory: 01 RF card

Installation Method
1. Remove the column from the outer package of the column kit and check the column number. Remove the sealing plug.
2. Open the lid of column oven and connect the column to the pump side according to the flow direction indicated by the arrow on the label.
3. Tap the Rinse button.
4. Check the liquid supply pressure and ensure that there is no liquid leakage around the connection part of the column.
5. Close the cover of column oven and activate the lock.
6. Insert the RF card into the card reading slot of the Vercentra HB-20 analyzer.
7. Test samples according to the Vercentra HB-20 Analyzer Instruction Manual.

Stability . The column remains stable until the expiration date indicated on the label when the original packaging is not opened. It must be stored away from light, at a temperature between 2-8°C and should not be frozen. When installed in the equipment, the column has the same validity period indicated on the product label.

Performance characteristics

1. Appearance: The outer packaging should be neat and clearly labeled. The label should have an arrow indicating the flow direction of the liquid, and the outer wall should be free of burrs and foreign matter.
2. Column pressure range: 1.0 MPa to 14.0 MPa.
3. Repeatability: A sample with a percentage between 4.0%～6.5% of HbA1c was determined 20 times using the same batch of product. A CV ≤ 2.0% was obtained.
4. Accuracy: Two reference samples were determined 3 times using three batches of the product. The relative deviation between the target value and the found value was ≤ 6.0%.
5. RF card value: RF card value is 800 tests, 1600 tests or 2400 tests.
6. Interferences: Hemoglobin variants can be detected. HbA1c results cannot be affected by the presence of HbC, HbD, HbE and HbS in the

Precautions and warnings

1. Please read the Vercentra HB-20 analyzer label and manual before using the column carefully before use.
2. Do not use an expired column kit.
3. Do not mix batches of products. The column lot number must be used together with the corresponding reagent kit and hemolyzer.
4. When installing the column, ensure that the flow of reagents is in the same direction as the arrow indicated on the label of the column.
5. After changing the column, wash through the sample activation test until the resulting chromatograph is normal.
6. When the operation of the column is stopped for more than 1 week, the column should be removed and both ends of the removed column should be sealed with sealing plugs. The column should be stored at 2 ºC-8 ºC in the dark.
7. Carefully handle the column and do not strike or shake the column.

References

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Presentation

Product	Reference	Contents	
HbA1c Reagent Kit Hb-20 - Ref. 6001	6001-800	R A	4 x 500 mL
		R B	2 x 500 mL
		R C	2 x 500 mL
		R D	2 x 500 mL

For information on other commercial presentations, please visit www.labtest.com.br or contact Customer Service.

Consumer information

[Warranty Terms and Conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label provided that the procedures and storage conditions indicated on the label and in this insert have been followed correctly.

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro . Symbols used with IVD devices

Conteúdo suficiente para < n > ensaios Contenido suficiente para < n > ensayos Sufficient content for < n > trials	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	**Produto de uso único** Producto de un solo uso Single use product	**Risco biológico** Riesgo biológico Biological risk
Prazo de validade (aaaa-mm-dd ou mm/aaaa) Fecha de expiración (aaaa-mm-dd o mm/aaaa) Expiration date (yyyy-mm-dd or mm/yyyy)	**Período após abertura** Período post-abertura Period after-opening	**Fabricante** Fabricante Manufacturer	**Corrosivo** Corrosivo Corrosive
Limite de temperatura (conservar a) Temperatura limite (conservar a) Temperature limit (store at)	**Número do catálogo** Número de catálogo Catalog Number	**Data de fabricação** Fecha de fabricación Date of manufacture	**Tóxico** Tóxico Poison
Representante Autorizado na Comunidade Europeia Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	**Identificador único do dispositivo** Identificador único del dispositivo Unique device identifier	**Uso veterinário** Uso veterinario Veterinary use	**Marca CE** Marcado CE CE Mark
Carcinogênico/mutagênico e/ou sensibilizante à respiração Carcinogénico/mutagénico y/o sensibilizante respiratorio Carcinogenic/mutagenic and/or respiratory sensitizer	**Número do lote** Denominación de lote Batch code	**Controle** Control Control	**Atenção** Atención Attention
Tóxico para os organismos aquáticos Tóxico para los organismos acuáticos Toxic for aquatic organisms	**Gases/líquidos comburentes** Gases/líquidos oxidantes Oxidizing gases/liquids	**Controle negativo** Control negativo Negative control	**Liofilizado** Liofilizado Lyophilized
Reagente contendo micropartículas Reactivo con microparticulas Reagent with microparticles	**Substância inflamável** Sustancia inflamable Flammable substance	**Controle positivo** Control positivo Positive control	**Instalar até** Instalar hasta Install before
Dispositivo médico de diagnóstico in vitro Dispositivo médico para diagnóstico in vitro In vitro diagnostic medical device	**Material Calibrador/Padrão** Material Calibrador/Estándar Calibrator/Standard Material	**Reagente** Reactivo Reagent	

Ref.: 260624