GLYCATED HEMOGLOBIN A1

Spectrophotometric Determination of Hemoglobin A1 (HbA1) in whole blood
Ion-exchange method in tubes

40 tests **REF KR13-40**

INTENDED USE

Kit for quantitative determination of Glycated Hemoglobin A1 in blood samples.
Hemoglobin is a protein within red blood cells that transports oxygen through the body. When it joins with glucose in the blood, it becomes glycated. The higher a person's blood glucose levels have been, the higher the number of red blood cells that will have become glycated.
By measuring glycated hemoglobin, it is possible to get an overall picture of what the average blood sugar levels have been over a period of weeks/months.

PRINCIPLE

Mixing hemolyzed whole blood with an ion-exchange resin, non-glycated hemoglobin fraction binds to the resin. A filter separates the resin from the supernatant containing the glycated hemoglobin. The percentage of glycated hemoglobin (HbA1) is determined by measuring the absorbances of the fractions of glycated hemoglobin and total hemoglobin against a calibrator.

REAGENTS

For in vitro diagnostic use only.

Kit components		REF KR13-40
Reagent 1	hemolyzing reagent	**1x20 mL**
Test Tubes	tubes with resin	**40 pcs**
Filters tubes	filters	**40 pcs**
Calibrator lyo	see values on the label	**2 x 0.5 mL**

STABILITY: reagents and tubes are stable up to the expiry date printed on the label if stored at 2-8°C and contamination is avoided. Keep the reagents closed when not in use.

REQUIRED BUT NOT PROVIDED

Spectrophotometer or filter photometer.
Timer.
Pipette and tips.
Distilled water.
Shaker.
Standard laboratory equipment.

SAMPLE

Whole blood in EDTA.
STABILITY: 7 days, if stored at 2-8°C.

PREPARATION OF THE CALIBRATOR

Reconstitute the content of one vial of Calibrator in 0.5 mL of cold distilled water. Shake gently by inversion till it is completely dissolved. Perform the procedure quicky so the calibrator does not reach room temperature. Put in fridge immediately.
STABILITY: reconstituted calibrator is stable 2 weeks at 2-8°C. Do not bring it to room temperature, take from the fridge the quantity needed to run the test (100 µL) and use it immediately.
If the calibrator won't be used within two weeks, pour 100 µL in Eppendorf tubes with cap and freeze at -20°C.
STABILITY: 8 weeks. Thaw and use it immediately.
Do not use if turned to brown or turbid.

ASSAY PROCEDURE

Wavelength	415 nm
Optical path	1 cm
Reading	Against distilled water
Temperature	18-30°C
Method	Spectrophotometric

PREPARATION OF THE HEMOLYSATE

Pipette into tubes labeled for samples and calibrator:

	Hemolyzed sample	Hemolyzed calibrator
Sample	100 µL	---
Calibrator	---	100 µL
Reagent 1	500 µL	500 µL

Mix well and incubate for 10 minutes at room temperature.

SEPARATION OF THE GLYCOSYLATED HEMOGLOBIN

Pipette 100 µL of hemolyzed sample and 100 µL of hemolyzed calibrator in the tubes with resin supplied with the kit properly labeled (one tube for each sample and/or calibrator).
Place the filters inside the tubes at approx. 2 cm from the liquid. Shake 5 minutes on a rotator or by inversion.
Let the resin deposit and push the filter to press the resin on the bottom.
Pour the supernatant in cuvettes (one cuvette for each sample and/or calibrator) and read the absorbance of the sample and of the calibrator (AHb GLIC) against distilled water within one hour.

PREPARATION OF TOTAL HEMOGLOBIN

Pipette in labeled tubes:

	Total Hemoglobin Sample	Total Hemoglobin Calibrator
Hemolyzed sample	20 µL	---
Hemolyzed calibrator	---	20 µL
Distilled water	5000 µL	5000 µL

Shake the tubes and read the absorbances against distilled water (TOTAL AHb).

CALCULATION

Calculate the ratio (R) of the absorbance of glycated hemoglobin and total hemoglobin of both sample and calibrator. Calculate the concentration of the sample according to the following equation:

$$R\ (\text{Sample Ratio}) = \frac{\text{AHb GLIC (Sample)}}{\text{AHb TOTAL (Sample)}}$$

$$R\ (\text{Calibrator Ratio}) = \frac{\text{AHb GLIC (Cal)}}{\text{AHb TOTAL (Cal)}}$$

$$\%\ \text{Sample Glyc. Hemoglobin} = \frac{R\ (\text{Sample})}{R\ (\text{Cal})} \times \%\ \text{Cal}$$

<u>Calculate R calibrator value for each series of samples to avoid interferences related to the temperature</u>.

REFERENCE VALUES

HbA1

Normal: 6.0 - 8.3%
Pathologic: > 10.0%
Normal values may vary depending on age, gender, diet, geographical location and other factors, therefore each laboratory should establish its own reference range.

DETERMINATION OF HbA1c

The values of HbA1 can be converted to HbA1c according to the following formula:
HbA1c value = (0.838 x HbA1 value) - 0.732
To obtain the value of HbA1c directly, use Calibrator percentage value printed on the label of the vial.

HbA1c

Normal: 4.2 - 6.2%
Pathologic: > 7.6%

ESTIMATED AVERAGE GLUCOSE OF BLOOD

The average glucose in blood (eAG) of patients frequently tested can be calculated in a 6-8-weeks range (in 6.5-13.0% range), using the following formula:
eAG (mg/dL) = (36.7 x HbA1 value) - 185

QUALITY CONTROL

Each laboratory should implement a quality control program.

PERFORMANCES

Limitations: not known limitations.
Linearity: 4.0% - 17%.
Within-run precision: CV 2.4%
Between-run precision: CV 5%
Interferences: high lipemia may cause false high HbA1 results. If this occurs, centrifuge the blood and resuspend red cells in a volume of saline solution equal to the original plasma, then run the test again.

PRECAUTIONS

Do not mix reagents from different lots.
Handle the reagents carefully, avoid ingestion and contact with skin, eyes and mucous membrane.
The reagents must be used only for the intended purpose and according to good laboratory practices.
Procedures for manipulation of the reagents are reported on MDSD (available upon request).
A correct diagnosis should never be made solely on the basis of a single test, but it has to be integrated with further clinical information.
Room temperature, temperature of the working reagents, accuracy of the washing steps and the type of the spectrophotometer may affect the performances of the test.

NOTES

1. Dilute with saline solution blood samples with total hemoglobin higher than 18 g/dL and multiply the results for the dilution factor.
2. Unstable fractions of hemoglobin in the sample are removed by the resin.
3. HbF does not interfere significantly.
4. Glycated HbS and HbC bind to the resin and may cause false normal values.

DISPOSAL

Safe disposal of kit components must be according to local regulations.

BIBLIOGRAPHY

Mayer and Freedman (Clin. Chim Acta 1983; 127:147-184)

SYMBOLS

LOT	Batch code		Refer to supplied instructions		Refer to supplied documentation		Manufacturer	CE	European Conformity
REF	Catalogue number		Use by (year/month)		Keep dry		Don't reuse	NON STERILE	Non-sterile
IVD	In vitro diagnostic		Temperature limitation		Keep away from heat and sunlight		Fragile		

IVD CE Ed. 04 – 03.2024 RR

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