GLYCOHEMOGLOBIN (HbA_1)

Colourimetric and quantitative determination of glycohemoglobin by chromatographic method in test-tube

TEST SUMMARY

The red corpuscles are lyzed by a reagent that, at the same time break the failing fraction of glycohemoglobin. The glycosated hemoglobin (HbA_1) is not bound to the resin put into the tube, remaining in the supernatant, while the other hematic components are absorbed by it. By means of a filter, HbA_1 is been separated and it is read in a photometrical way in comparison the total hemoglobin.

SAMPLES

Whole blood with EDTA as anticoagulant.
Stability: 7 days at 2-8°C.

REAGENTS

Chromatographic tubes
Ion exchange resin into buffer pH 6.9 - 7.2.

Hemolysing reagent
Lysing solution containing ion borate and derivants of cyanide.

Total hemoglobin
Test tubes containing the diluent for total hemoglobin.

Standard
Lyophilized blood knowed as HbA_1.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GPL) guidelines.

WARNING: the lysing solution contains derivants of cyanide, avoid the contact with skin and eyes; in case of contact wash carefully with water and consult a doctor.

REAGENTS PREPARATION

All reagents are ready to use and stable until expiration date on the label, stored at room temperature.
Reconstitute the standard with 1 ml of distilled water, let stand 30 minutes before use.
Stability: 15 days at 2-8°c or 8 weeks at –20°C.

SAMPLE PREPARATION

Add 100 µl of whole blood well mixed in a tube that contains hemolysing reagent. Mix carefully and let stand at least 5 minutes.
Do the same for the standard.

PROCEDURE

Add 100 µl of hemolized in the tube that contains resin, introduce the filter at approximately 1 cm over the liquid surface.
Shake for 5 minutes on vortex or in alternative continue in exchanging them for the same time.
Then press carefully the filter against the bottom of the tube and decant the supernatant and read the absorbance.

At the same time prepare the total hemoglobin solution adding 10 µl of hemolyzed into the tubes contained diluent for the total hemoglobin.

PHOTOMETRIC READING

Read at 415 nm (within 1 hour) extinction of the surnatant and of the total hemoglobin solution of each sample bring to zero with distilled water.

CALCULATION

$$\text{Factor} = \frac{(A_{\text{total hemoglobin standard}})}{(A_{\text{fraction non-absorbed standard}})} \times \text{standard value}$$

$$\%\ \text{Glycohemoglobin} = \frac{(A_{\text{fraction non-absorbed}})}{(A_{\text{total hemoglobin}})} \times \text{factor}$$

EXPECTED VALUES

Glycohemoglobin: 6.0 – 8.3 %

Each laboratory should establish appropriate reference intervals related to its population.

NOTE

- Operate at a temperature between 21 – 26 °C.
- The use of control blood is suggested if the range of temperature won't be respected.
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control.

TEST PERFORMANCE

Precision

Intra-assay CV%:	2.23
Inter-assay CV%:	2.68

Limitations
High value of faetal hemoglobin (HbF) will give abnormally high HbA1 values.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC01950	**(50 TESTS)**
Tubes with resin	50 x 3 ml
Hemolysing reagent	50 x 0.5 ml
Tubes for total hemoglobin	50 x 2.5 ml
Standard	1 x 1 ml
Filter	50

CODE CC01960	
Standard	1 x 1 ml

REFERENCES

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Nathan, D. M., Singer, D.E., Hurxthal, K., Goodson, J.D. (1984). New Engl.J.Med., 310, 341-346.

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SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biological risk

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