

DETERMINATION OF GLYCOSYLATED HEMOGLOBIN

Introduction

The normal adult hemoglobin contains 90% hemoglobin A (HbA), which is a tetramer of two α - and two β globin chains. Both subunits contain one hem prosthetic group. A minor form among the hemoglobins is the glycohemoglobin (HbA1), the glycosilated form of HbA. The most common form is the HbA1c, where one unit of glucose is coupled to the N terminal valine of the β globin chain.

The glycosilation of HbA1 occurs non enzymatically during the whole lifespan of the erythrocytes. Normally 4.5-6.5 % of the hemoglobin is glycosilated, but in diabetes this value can be 2-3 times higher.

Although the exact clinical significance of glycosilated hemoglobins is still not clear, the measurement of the HBA1 level is important because its amount is rational to the long-term average glucose concentration, and especially because its amount is not influenced by the patient's meal, physical activity, and/or the antidiabetic drugs taken before drawing the blood sample.

The rationale of the method

The determination of glycosilated hemoglobin is conducted according to the Flückiger and Winterhalter method. The glucose bound to the primer amino groups of hemoglobin can be removed by acid catalysis and heat in the form of 5-hydroxi-methyl-furfural. This product gives a color reaction with thiobarbituric acid.

Reagents:

- 1.Solution for hemolysis: 0.1 %-Triton X-100
- 2.Arsenate-sulphuric acid solution: 0.01 disodium-hidrogen-arsenate dissolved in 0.3 N sulphuric acid.
- 3.Trichloro-acetic acid solution: 40 % TCA in d.w.
- 4.Thiobarbituric acid solution: 0.025 M thiobarbituric acid dissolved in 0.01 M sodium hydroxide.

Sample preparation

5.0ml of whole blood anticoagulated with heparin was centrifuged, and after removal of the plasma the cell pellet was washed three times with physiologic buffer. The red blood cells were hemolysed with one and a half volume of hypotonic buffer.

We are working here with ready made hemolysed serums.

Experimental procedure

Pipette into the long test tube:

	blank	normal sample	pathologic sample
	1	2	3
Haemolysed serum		1.0 ml	1.0 ml
Water	1.0 ml	-	-
Arsenate sulphuric acid	0.5 ml	0.5 ml	0.5 ml

Mix the tubes and incubate for 50 minutes in boiling water. After the incubation cool the solutions to 30-40 °C:

Add 0.5 ml TCA solution to each tube **drop by drop** while continuously mixing with vortex.

Centrifuge the mixture at 10 000 RPM for 5min. in Eppendorf tubes.

Take into the normal test tubes:

Supernatant	1.0 ml	1.0 ml	1.0 ml
add			
Thiobarbituric acid	0.25 ml	0.25 ml	0.25 ml

Mix the tubes and incubate for 35 minutes at 40 °C, then cool to 20 °C.

Measure the extinction of the samples at 433 nm against the blank in a narrow cuvette.

Calculations

$$\text{HGb} = \frac{E}{0,006 * \text{Hg}\%}$$

Where 0.006 = dilution * extinction coefficient

Normal values: 4.5-6.5 % GHb% Depending on the kit the values might be different.

Sample	GHb %
Normal serum	
Pathologic serum	