GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PDH)

Quantitative UV determination of glucose-6-phosphate dehydrogenase in the blood

CLINICAL SIGNIFICANCE

Glucose-6-phosphate dehydrogenase deficiency is very common in pathologies that lead enzymopathies. This deficiency derived from a recessive hereditary character linked to X chromosome and can cause high red blood cell destruction, up to anemia.

TEST SUMMARY

Glucose-6-phosphate dehydrogenase catalyzes the first reaction phase in pentose phosphate shunt, by oxidizing glucose-6-phosphate in 6-phosphogluconate and reducing NADP in NADPH.
The rate of formation of NADPH is proportional to the G-6-PDH activity and the absorbance increase can be measured spectrophotometrically at 340 nm.

SAMPLES

Collection of samples according to CLSI (NCCLS). Whole blood treated with ACD (citric acid dextrose), EDTA or heparin. The G-6-PDH enzyme of red blood cells in whole blood is stable for 7 days at 2-8 ° C. However, it is unstable in the hemolysate. It is advised not to freeze the blood.
Since G-6-PDH activity is expressed in units relative to the amount of red blood cells and / or units relative to grams of hemoglobin, red blood cell counts and / or hemoglobin concentration should be determined before Test.
Samples taken in ACD do not gives stability problems for red blood cell counts, unlike samples taken in heparin, for which it is advised to report the G-6-PDH activity results in grams units of hemoglobin.

REAGENTS

Reagent 1: Buffer pH 7.6, ions Mg, G-6-PD, preservatives and stabilizers.

Reagent 2: Buffer pH 6.7, lysing agent, NADP, Maleimide, preservatives and stabilizers.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvettes in optical glass or disposable in optical polystyrene. Distilled water. Reagents for the determination of the hemoglobin or instruments for the counting of erythrocytes.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Closed Reagents are stable until the expiration date indicated on the labels stored at 2-8°C.
Reagent 1 is ready for use.
Reconstitute a Reagent 2 vial with 5.5 mL of distilled water by mixing until complete solubilization.
The reconstituted Reagent 2 is stable for 7 days at 2-8°C.

PROCEDURE

Kind of analysis.:	Kinetic (Increasing)
Reading time:	12 minutes, 17 minutes
Wavelength:	340 nm
Temperature:	37°C*
Zero:	Distilled water

* The reaction temperature can be changed.
During the calculation, apply the TFC Factor – Correction of Temperature based on the temperature used.

Reagents	Standard	Sample
Reagent 2	1 mL	1 mL
Standard	10 µl	--
Sample	--	10 µl
Shake gently and allow to stand at 37°C for 10 minutes. Then add:		
Reagent 1	2 mL	2 mL

Mix well and incubate at 30°C for 2 minutes.
Read the absorbance A1 against water.
Incubate at 30°C for 5 minutes after the first reading and read the absorbance A2 against water

CALCULATION

Calculate ΔA for min. = (A2-A1) / 5

The activity of the G-6-PDH is expressed as:
U/10^{12} erythrocytes (RBC) or **U/g of hemoglobin (Hb)**

G-6-PDH (U/10^{12} RBC) =

$$\frac{\Delta A \text{ for min.} \times 3.01 \times 10^{12} \times TCF}{0.01 \times 6.22 \times (N \times 10^{6}) \times 1000}$$

where:
3.01 = Total reaction volume
10^{12} = Factor to express the activity in 10^{12} of cells
0.01 = Sample Volume (mL)
6.22 = NADPH millimolar absorbivity at 340 nm
$Nx10^6$ = Calculation of red blood cells (red blood cells /mm^3) determined for each sample
1000 = Erythrocyte count conversion from mm^3 to mL
TCF = Temperature conversion factor (at 30°C = 1)
(See the table at the end of the paragraph)

The equation is reduced to:

$$\textit{G-6-PDH (U/10}^{12}\textit{ RBC)} = \frac{\Delta A \text{ for min.} \times 48390}{N} \times TCF$$

where:
N = Erythrocyte count divided by 10^6
TCF = Temperature conversion factor (at 30°C = 1)

G-6-PDH (U/g Hb) =

$$\frac{\Delta A \text{ for min.} \times 100 \times 3.01 \times TCF}{0.01 \times 6.22 \times Hb\ (g/dL)}$$

where:
3.01 = Total reaction volume
100 = Conversion factor of the activity in 100 mL
0.01 = Sample Volume (mL)
6.22 = NADPH millimolar absorbivity at 340 nm
Hb (g/dL) = Hemoglobin concentration of sample
TCF = Temperature conversion factor (at 30°C = 1)

The equation is reduced to:

$$\textit{G-6-PDH (U/g Hb)} = \frac{\Delta A \text{ for min.} \times 4839}{Hb\ (g/dL)} \times TCF$$

where:
Hb (g/dL) = Hemoglobin concentration of sample
TCF = Temperature conversion factor (at 30°C = 1)

EXAMPLES

A sample with erythrocyte count of 4.4 x 106 and Hemoglobin concentration of 14.8 g/dL gave a ΔA/min at 30°C of 0.033

$$\textit{G-6-PDH (U/10}^{12}\textit{ RBC)} = \frac{0.033 \times 48390}{4.4} = 363$$

$$\textit{G-6-PDH (U/g Hb)} = \frac{0.033 \times 4839}{14.8} = 10.79$$

CALIBRATION

The procedure is standardized based on the millimolar absorptivity of NADPH which is 6.22 to 340 nm.

REFERENCE VALUES

146-376 U/10^{12} RBC
4.6-13.5 U/g Hb

In infants the values may be slightly higher.
Since normal values depend on many factors (age, sex, diet, geographic area, etc. ..), each laboratory should, however, establish appropriate reference intervals related to its population.

NOTES

- As with any diagnostic procedure, if the results are incompatible with clinical presentation, the physician should confirm the data obtained using this test, with other clinical information.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control.

TEST PERFORMANCE

Under evaluation

WASTE DISPOSAL

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

PACKAGING

CODE CC04200	(50 TESTS)	
Reagent 1	2 x 50 ml	(liquid)
Reagent 2	10 x 5.5 ml	(liophile)

REFERENCES

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SYMBOLS

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

NOTE: MODIFICATIONS HIGHLIGHTED WITH GRAY BACKGROUND