QUANTON BIOLIFE SCIENCES

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G6PD (Quantitative) Test Kit

Photometric

QBL/PDS/GPQ_018

Quantitative determination of G6PD (Quantitative) in human whole Blood
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Pack Size
GPQ 10	1 X 10 Test
GPQ 25	1 X 25 Test
GPQ 50	1 X 50 Test
GPQ 100	1X100 Test

CLINICAL SIGNIFICANCE

Glucose-6-Phosphate-Dehydrogenase (G6PD) deficiency is one of the most common human enzyme deficiency in the world. During G6PD deficiency, the red cells are unable to regenerate reduced Nicotine adenine dinucleotide phosphate (NADPH), a reaction that is normally catalyzed by the G6PD enzyme. Since the X chromosome carries the gene for G6PD enzyme, this deficiency mostly affects the males.

The two major conditions associated with G6PD deficiency are hemolytic anaemias and neonatal jaundice, which may result in neurological complications and death. Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counselling and abstinence from disease precipitating drugs such as anti malarials and other agents

Method

Photometric test Method.

PRINCIPLE

Glucose 6 phosphatase dehydrogenase catalyzes the first step in the pentose phosphate shunt , oxidizing glucose-6- phosphate to 6 phosphogluconate and reducing NADP to NADPH. The accucare procedure is a spectrophotometric method based on the following reaction

G6P+ NADP+ ----------→ 6-PG + NADP + H+ NADP

is reduced by G-6PDH in the presence of G6P. The rate of formation of NADPH is proportional to the G-6-PDH activity and is measured spectrophotometrically as an increase in absorbance at 340 nm Production of a second molar equivalent of NADP by erythrocyte 6-phosphogluconate dehydrogenase.

6-PG+NADP+ ----------→ Ribulose-5-Phosphate + NADPH +H+ CO2

Is prevented by use of maleimide and inhibitor of 6-PGDH.

REAGENT

Reagent 1 : Single assay vials
Reagent 2 : Diluent Reagent
Reagent 3 : Substrate reagent

REAGENT PREPARATION

Bring all the reagents to room temperature .Tap the substrate vials gently on a flat surface to dislodge all the substrate powder. Just before use using clean pipette reconstitute each substrate vial with the volume of diluent reagent as stated on vial. Gently swirl to dissolve and allow to stand for 5 minutes.

REAGENT STORAGE AND STABILITY

Store unopened G-6-PDH reagent vials and the G-6-PDH substrate solution in refrigerator. Reagents are stable until expiration dates shown on the labels. Reconstituted G-6-PDH assay solution is stable for 8 hrs at room temperature or 5 days refrigerator.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Whole Blood with EDTA/Heparin

Red cell G6PDH is stable in whole blood for 1 week at 2 – 8 °C, but is unstable in red cell hemolysate. Freezing of blood is not recommended.

G6PDH is very unstable in hemolysates. 20/30 minutes after dilution a precipitate may appear, probably due to the biological variability of the patient's sample.

Since activity is reported in terms of number of red cells or grams of hemoglobin.The red cell count or hemoglobin concentration should be determined prior to performing of G-6-PDH assay. The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts poses no problem. However red cell counts on specimens collected in heparin become unreliable after about 2 days. Thus for heparinized sample results are best reported in terms of hemoglobin concentration.

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, Calibrator and samples to room temperature 18 - 28ºC, prior to analysis.

AUTOMATED PARAMETERS	
Wavelength	340 nm
Measurement	Against Distilled Water
Cuvette	1 cm light path
Reaction Temperature	Room Temperature
Reaction Type	Kinetic
Reaction Direction	Increasing
Sample Volume	10 µl (0.01 ml)
Reconstituted Assay Reagent Volume	1.0 ml
Substrate Reagent Volume	2.0 ml
Delay/Lag/time	300 secs
Interval time	60 secs
No. of Readings	05
Factor	4839
Low Normal at 37°C	4.6 u/g Hb
High Normal at 37°C	13.5 u/g Hb
Linearity at 37°C	19.5 u/g Hb

MANUAL ASSAY PROCEDURE

The temperature of the reaction mixture should be maintained at 30 °c or some other constant temperature.

Addition Sequence	ML (µl)
Reconstituted Assay Reagent	1.0 ml (1000 µl)
Sample	0.01 ml (10 µl)
Mix well and incubate for 5 mins at 30°C & Immediately Add	
Substrate Reagent	2.0 ml (2000 µl)
Mix well and After 5 minutes read the absorbance (A_0) & repeat the absorbance reading after every 1,2,3,4,&5 min. Calculate Mean absorbance Change per min. (ΔA per min)	

SAMPLE DILUTIONS

- This method is linear upto a concentration of 19.5 U/G Hb.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

CALCULATION

G6PDH activity is expressed as $U/10^{12}$ erythrocyte or U/G hemoglobin (Hb)

$$\text{G6PDH } (U/10^{12}\ \text{RBC}) = \Delta A \text{ per min} \times \frac{48390}{N} \times TCF$$

Where N= Red cell count divided by 106
TCF = temperature correction factor (1 at 30 ºC)

$$\text{G6PDH (U/G Hb)} = \Delta A \text{ per min} \times \frac{4839}{\text{Hb(g/dL)}} \times TCF$$

UNIT DEFINATION

One international unit is that amount of G6PDH activity that will convert 1 micromole of substance per minute under the conditions specified in the insert. Activity may be expressed in terms of either a standard no of cell or amount of hemoglobin. Since it is preferred despite the fact that it is believed by some that red cell counts are subject to considerable uncertainity. Hemoglobin concentration may be determined with greater accuracy but the amount of hemoglobin contained in a cell is under separate genetic control and may wary independently of G6PDH activity.

CLIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable calibrator is recommended.

The procedure is standardized on the basis of the millimolar absorptivity of NADPH which is 6.22 at 340 nm. The oxidative conversion of G6P by G6PDH leads to reduction of NADP to NADPH on a molar equivalent basis.Measurement of the rate of increase in Absorbance at 340 n serves to quantitate enzymatic activity. The maximum G-6-PDH activity which may be measured by this procedure is approximately 650 U/10 RBC or 19.5 U/GHb.

It is recommended to run a normal and a pathological control which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Control Low	237.51	0.33	0.14%
Control Normal	1305.61	0.40	0.03%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Control Low	237.05	0.24	0.10%
Control Normal	1305.13	0.03	0.00%

LINEARITY

This method is linear upto a concentration of 19.5 U/G Hb. Dilute samples above this concentration 1:1 with 0.9% saline. Repeat assay. Multiply the result by 2.

METHOD COMPARISON

A comparison of G6PD (Quantitative) with a commercially available assay (x) using 20 samples gave following results: $R^2 = 0.9900$

REFERENCE VALUES

The following range of G6PDH values measured at 30°C was obtained in our laboratory for 100 clinically healthy males and females.

G6PDH Activity
146 - 376 ($U/10^{12}$ RBC)
4.6 - 13.5 (U/g Hb)

Values for newborn may range somewhat higher. It is recommended that each laboratory establishes its own normal range.
It has been determined that G6PDH deficiency in red cells is the basis for certain drug induced hemolytic anemias. This type of susceptibility to drug induced hemolysis is often called “primaquine sensitivity” because studies which led to its characterization were made during investigations of the hemolytic properties of this antimalarial compound.

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

INTERFERENCE

Both copper, which completely inhibits the enzyme at a concentration of 100 mmol/L and sulfate ions (0.005 mol/L) will decrease observed values of G-6-PDH activity, certain drugs and other substances are known to influence circulating levels of G-6-PDH.
Reticulocytes have higher G-6-PDH levels than mature red cells. Therefore it is not recommended that assay be performed after a severe hemolytic crisis, since G-6-PDH levels appear falsely elevated. Under those conditions detection of deficiency may require family studies. Testing may be more helpful after the level of mature red cells has returned to normal. Under normal circumstances activity contributed by leucocytes, platelets and serum is relatively small. However in cases of extreme anemia, grossly elevated white counts or very low levels of red cell G-6-PDH activity, the contribution to the total made under these circumstances may be significant.

Use of Buffy Coat-Free Sample

Under normal circumstances g6PDH activity contributed by leucocytes, platelets an serum is relatively small. However as reported by Echler and others more accurate measurement of G6PDH activity specially in presence of anemia and or leucocytosis can be achieved by using buffy coat free blood samples for assay. Thus in case of a borderline value obtained with whole blood it may be warranted to repeat the assay on a buffy coat free sample.

BIBLIOGRAPHY

Burtis, C.A., Ashwood, E.R., Tietz Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, pp. 1645-1650, 1999.
Kornberg, A., Horecker, B.L.: Glucose-6-Phosphate Dehydrogenase. IN Methods in Enzymology. S.P. Colowick, N.O. Kaplan, Editors, Vol. I, Academic Press, New York, p 323, 1955.

GLOSSARY OF SYMBOL

	Consult Instruction for Use
	Catalog Number
	Store between
	Manufacturer
	Keep away from sunlight

Paramcare Life Sciences Private Limited, G/F-12/13, Evershine-2,
Survey No. 307/3/1, Balitha N.H No 48, Vapi, Valsad, Gujarat, 396191.

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