GLUCOSE-6-PHOSPHATE DEHYDROGENASE

HEMOPAK

(Qualitative Visual)

Last update 09-2020

Ref. CC1-GPD.09M, 12Test

INTENDED USE

Glucose-6-Phosphate Dehydrogenase qualitative test for screening G6PD deficiency in erythrocytes.

PRODUCT HIGHLIGHTS

- Perhaps only company in India making both visual (dye) and UV assay reagents.
- Reagent ensuring complete inhibition of 6-Phosphogluconate Dehydrogenase, thereby giving better accuracy.
- Very convenient pack sizes.
- Giving additional reagent for running a control in dye decolourization.
- Q.A. procedures to ensure very high quality, high precision.
- Use of lysing reagent.

PRINCIPLE

Glucose-6-Phosphate Dehydrogenase (G6PD) present in hemolysate acts on substrates, Glucose-6-Phosphate ($G6PO_4$) and NADP, giving NADPH which in presence of PMS decolourises blue coloured indophenol dye (DCPIP) leaving behind colour only due to hemolysate. The rate of reaction being proportional to enzyme activity (G6PD) present, time required for decolourization is inversely proportional to enzyme activity in the hemolysate.

The reaction is as shown.

Glucose-6 Phosphate + NADP $\xrightarrow{\text{G6PD}}$ 6-Phosphogluconate + NADPH

NADPH + DCPIP $\xrightarrow{\text{PMS}}$ Reduced DCPIP

PRESENTATION

Store all reagents at 2-8^0 C	No. of Bottles 12 Test
• G6PD-1 (Co-enzyme Substrate)	13
• G6PD-2 (Buffer)	1
• G6PD-3 (Lysing Reagent)	1
• G6PD-4 (Inert Oil)	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Glucose-6-Phosphate	10 mmol/L
• NADP Na_2	1.5 mmol/L
• DCPIP	0.5 mmol/L
• Buffer	100 mmol/L
• Detergent	1 mmol/L
• Inert Oil	-
• pH 8.0 $\pm$ 0.1 at 25^0 C	

Also contains non-reactive fillers and Stabilizers.

PRECAUTION

1. G6PD is for *in-vitro* use only.
2. Use clean and dry glassware, G6PD-3 should be cold (4^{0}C) before use.
3. Fresh whole blood should be used as the enzyme activity decreases on storage at 2-8^{0}C.
4. Always run one control sample of a known, healthy nondeficient subject on opening a kit.
5. Do not disturb the vial after addition of inert oil as the disturbance will introduce air and oxidise the NADPH or dye which may lead to erroneous results.

PREPARATION OF WORKING REAGENT

Add 0.5 ml G6PD-2 (Buffer) to the Vial labelled G6PDH-1 (Co-Enzyme Substrate). Shake well to allow complete dissolution and use within 10 minutes.

REAGENT STORAGE AND STABILITY

G6PD reagents are stable until the expiry date stated on the label.

Reconstitute reagent G6PD-1, just before use.

SAMPLE COLLECTION

1. Whole blood should be collected using EDTA as an anticoagulant. Heparin should not be used as it interferes with the reaction.
2. Finger prick blood may be used provided the hemoglobin content is close to 15 gm%. For an unknown sample, the hemoglobin content must first be estimated and aliquot of blood may be corrected for low hemoglobin content.

TEST PROCEDURE

Estimate Hemoglobin content (gm/dl) of whole blood. If the Hemoglobin content is significantly less than 15 gm/dl, adjust the Hemoglobin content to 15gm/dl by proportionately increasing the aliquot of whole blood during preparation of red cell hemolysate.

1. PREPARATION OF RED CELL HEMOLYSATE

Given below is a table showing quantity of blood required for 1 ml lysing reagent corresponding to the Hemoglobin concentration Hb gm/dl.

Hemoglobin Concentration (gm/dl)	Quantity of blood to be taken (ml)
7.0 - 9.5	0.04
9.6 - 11.5	0.03
11.6 - 13.5	0.025
13.6 - 15.0	0.02

G6PD-3 (Pre-cooled lysing reagent)	1.0 ml.
Fresh Whole Blood	0.02 ml (Or refer table above)

Mix well and keep it in the refrigerator (2-4^{0}C) for 10-15 minutes and use as given below:

1. Transfer completely the red cell hemolysate to the freshly prepared working reagent and shake well.
2. Immediately overlay 1.0 ml. of G6PD-4 (Inert Oil).
3. Seal the vial tightly, using the plug and cap to make it air tight, incubate at 37^0 C.
4. Observe the change of initial blue to brownish colour.

2. OBSERVATIONS

Observe the reaction mixture at 30 minutes for decolourisation. If the decolourisation is incomplete, observe for every 5 minutes (or shorter intervals) thereafter until the decolourisation is complete. If the decolourisation takes longer time than 60 minutes, increase the interval time between observations and follow up for 4-8 hours or more.

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TEST RESULTS

1. In normal subjects, decolourization time is between 30-60 minutes.
2. In G6PD deficient subjects, (heterozygous males and homozygous females) decolourization time is between 2 to 24 hours.
3. In heterozygous females, who are carriers, the cell population is mixed with normal and deficient cells. The distributions of deficient cells varies from individual to individual, ranging from 20% to 80%. Hence some such subjects may give results overlapping over normal as well as abnormal time specifications i.e. the decolourization time in some heterozygotes will be between 30-60 min. (Normal) and for some heterozygotes the same will be 2 hours or more.

NOTES

1. Sample may give false normal result in a deficient subject if the recticulocyte count is high, as recticulocytes have a higher G6PD activity than adult red cells. This is of special importance if the test is carried out immediately after a hemolytic episode in a drug (primaquine or any such) sensitive subject.
2. After initial 15 minutes it is better to observe the reaction tube at an interval of 5 minutes or less as some of the sample may reach the end point and then slowly turn blue again, due to re-oxidation of the dye.
3. Observation of the colour change should be restricted to the reaction mixture below the layer of oil, and not at the interphase.
4. Vitamin C supplements or large amount of dietary intake of Vitamin C may interfere with the reaction.
5. To find out the G6PD activity of heterozygous males or females (carriers) it is advisable to estimate G6PD activity quantitatively, although mosaicism is better shown under microscope by Cytochemical Staining.

REFERENCE

1. BEUTLER, E, BLUME, K.G., KAPLAN, J.C. LOHAR, G.W. RAMOT, B. and VALENTINE, W.N. (1979) International Committee for Standardization in Hematology. Recommended Screening test for Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency. British Journal of Haematology, 43, 465.
2. DACIE V., LEWIS S., Practical Haematology, 7th Edition (1991) Pg. 204-212.

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