PATHOZYME DIAGNOSTICS

G6PDH

(QUALITATIVE / DYE DECOLOURIZATION METHOD)

CAT NO.: GPD

Reagent kit for qualitative test for screening G6PD deficiency in Erythrocytes.

DIAGNOSTIC SIGNIFICANCE:

Hemolytic anemias or hemolytic episodes are related in most of the cases to enzyme deficiencies due to hereditary abnormalities. There are many screenings nonspecific tests like osmotic fragility auto hemolysis tests etc. Additional better screening tests for metabolic defects in red cell are to measure glucose consumption, lactate production or measure contribution of pentose phosphate pathway to metabolism. However, these tests being elaborate and difficult and still not being specific, it is better to identify these deficiencies by enzyme assays.
One of the common enzyme deficiencies for hemolytic episodes / hemolytic anemia is measurement of Glucose-6-Phosphate Dehydrogenase, by a quantitative enzyme assay.
G-6-PD uses a potent inhibitor to prevent interference caused by 6-Phosphogluconate Dehydrogenase.

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.

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADPH} + H^+$$

$$\text{NADPH} + \text{DCPIP} \longrightarrow \text{Reduce DCPIP}$$

SPECIMEN COLLECTION:

Whole blood should be collected using EDTA as an anticoagulant. Heparin should not be used as it interferes with the reaction. Determine the **Hemoglobin content** of the whole blood prior to lysis of the cells.

KIT PRESENTATION:

PACK SIZE	10 Test	20 Test
R1-G6PD (Substate Reagent)	10 X 0.5 ml	20 X 0.5 ml
R2-G6PD (Buffer Reagent)	1 X 6 ml	1 X 11 ml
R3-G6PD (Lysing Reagent)	1 X 11 ml	1 X 22 ml
R4-G6PD (Inert Oil)	1 X 11 ml	1 X 22 ml

REAGENT PREPARATION AND STABILITY:

All reagents are stable at 2-8°C until the expiry date stated on the label.

PRECAUTIONS:

1. **R3-G6PD (Lysing Reagent) must be cold (2-8°C) before use.**
2. Fresh whole blood should be used as the enzyme activity decreases on storage 2-8°C.
3. Do not disturb the vial after addition of inert oil as the disturbance will introduce air and oxidize the NADPH or dye which may lead to erroneous results.

PREPARATION OF WORKING REAGENT:

Add 0.5 ml. R2-G6PD (Buffer Reagent) to the Vial labeled R1-G6PDH (Substrate Reagent). Shake well to allow complete dissolution and use within 10 minutes.
Reconstitution R1-G6PD (Substrate) just before use for test.

PROCEDURE:

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

PREPARATION OF RED CELL HEMOLYSATE:

Given below is a table showing quantity of blood required for 1 ml R3-G6PD (Lysing reagent) **(use cold)** corresponding to the Hemoglobin concentration Hb gm/dl.

Hb. Concentration (gm/dl)	Quantity to be taken (ml)
7.0 – 9.5	0.040 ml (40µl)
9.6 – 11.5	0.030 ml (30µl)
11.6 – 13.5	0.025 ml (25µl)
13.6 – 15.0	0.020 ml (20µl)

R3-G6PD (Lysing Reagent)	1000 µl (1.0ml)
Fresh Whole Blood	Refer above table as per Hb.

Mix well and keep it in the refrigerator (2-8°C) for 10-15 minutes and use as given below:

a) Transfer completely the red cell hemolysate to the **freshly prepared working reagent**. Shake well.
b) Immediately overlay approx. 1.0 ml. of R4-G6PD (Inert Oil). **Then do not shake or mix the all contents.**
c) Seal the vial tightly, using the rubber plug to make it air tight, incubate at 37°C and note the time.
d) Observe the change of initial blue to brownish colour.

(P.T.O.)

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Expiry Date | IVD In-Vitro Diagnostics Use | Storage | Mfg. Date | LOT Batch Number | REF Catalogue Number | See Package Insert

PATHOZYME DIAGNOSTICS
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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. In G6PD deficiency the time taken for decolourisation will exceed from 2 hours to 24 hours.

INTERPRETATION:

1. In normal subjects, decolourization time is between 5-60 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 vary 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 reticulocyte count is high, as reticulocytes 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 5 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 G6PDH deficiency. British Journal of Haematology, 43, 465.
2. DACIE V., LEWIS S., Practical Haematology, 7th Edition (1991).

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