Qualitative Screening test of Glucose-6-Phosphate dehydrogenase (G6PDH) in Whole Blood
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
G6PDS 10	10 x 1 ml

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

Coloration visual test Method.

PRINCIPLE

Glucose 6 phosphatase dehydrogenase present in the red cell hemolysate acts on glucose -6-phosphate and reduces NADP which in the presence of PMS reduces the blue colored 2,6 dichlorophenol Indophenol into a colorless form leaving behind the original cherry red color of the hemolysate. The rate of decolorisation is proportional to the enzyme activity.

REAGENT

Reagent 1 : Substrate Vials
Reagent 2 : Buffer Reagent
Reagent 3 : Mineral oil

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 0.5 ml of buffer reagent. Gently swirl to dissolve and allow to stand for 5 minutes.

REAGENT STORAGE AND STABILITY

Reagent 1 & 2 are to be stored at 2-8°C.All reagents are stable till expiry date mentioned on the vials when stored at proper storage conditions.
Reconstitute reagent G6PD-1, just before use.

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, standard and samples to room temperature 18 - 28°C, prior to analysis.

PREPARATION OF RED CELL HEMOLYSATE

Enclosed below is table showing amount og blood required to prepare the hemolysate corresponding to the Hemoglobin concentration (G/DL).

Hemoglobin concentration	Quantity of Blood (ml)
7.0 to 9.50	0.04 ml
9.6 to 11.50	0.03 ml
11.6 to 13.50	0.025 ml
13.6 to 15.0	0.02 ml

In 1.0 ml of D/W add 20 µl of well mixed EDTA whole blood sample. Mix well and allow to stand for 5 minutes at room temperature

MANUAL ASSAY PROCEDURE

- Add 1 ml of the hemolysate to the reconstituted substrate vial and mix gentle by swirling.
- Add immediately 1 ml of the mineral oil
- Replace the plug and the cap tightly. Incubate undisturbed at 37 °C for 60 minutes.

NOTE: The vials should be incubated undisturbed as the introduction of air from the atmosphere will allow the blue color to reappear and will give erroneous results.

INTERPRETION OF RESULTS

DECOLORISING TIME
Normal subject: upto 60 min.
G6PD deficient Subject (In heterozygous male and homozygous females) decolorisation time is 2-24 hrs.

In Heterozygous females who are carriers the cell population is mixed with normal and deficient cells. The distribution 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 specification i.e. the decolorisation in some heterozygous will be upto 60 minutes (normal) and for some heterozygous will be upto 2 hrs or more.

NOTES:

Samples having hemoglobin content below 15 gm% proportionally use more amount of blood for preparation of the hemolysate.
Blood with high reticulocyte count may give false normal even though the patient is enzyme deficient as reticulocyte, generally have a higher G6PD activity than adult red cell. This is of very

great importance if the test is carried out immediately after a hemolytic eposide in a primaquine sensitive subject.

CALIBRATORS AND CONTROLS

control serum 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

Diagnostic Sensitivity

Normal Specimen	Normal	Deficient	Sensitivity
	50	0	100%

Diagnostic Specificity

Deficient Specimen	Normal	Deficient	Specificity
	0	20	100%

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.

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.

BIBLIOGRAPHY

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.

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
Email: accucarediagnostics.com; Website: www.labcarediagnostics.com

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