ACCUCARE
G6PDH
Qualitative Screening method.

Qualitative Screening Test of 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.

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 COMPOSITION

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

SPECIMEN COLLECTION AND PRESERVATION

Use fresh blood samples as enzyme activity reduces on refrigeration. Do not use heparin as an anticoagulant Heparin interferes with the result.

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℃.All reagents are stable till expiry date mentioned on the vials when stored at proper storage conditions.

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 ℃ 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.

BIBLIOGRAPHY

S.K SOOD et al, The Indian Journal of Path and Micro 24 (1981),89.
Lubin, B.H. and Oski,F.A.,J. Pediatr. 70 (1967),788

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