G-SIX KIT

(Kinetic method)

For the determination of G6PDH activity in RBC's.
(For Invitro Diagnostic Use Only)

Summary

Glucose-6-Phosphate-Dehydrogenase (G6PDH) deficiency is one of the most common human enzyme deficiencies in the world. During G6PD deficiency, the red cells are unable to regenerate reduced Nicotineamide 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

G6PDH in the RBC's is released by a lysing agent present in the reagent. The G6PDH released catalyzes the oxidation of Gluose 6 phosphate with the reduction of NADP to NADPH. The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to the G6PDH activity in the sample.

$$\text{G - 6 - P + NADP} \xrightarrow{\textit{G - 6 - PDH}} \text{Gluconate - 6 - P + NADPH + H}$$

Normal reference values

G6PDH Activity (U/g Hb.) : 4.6 to 13.5 at 30°C / 6.4 to 18.7 at 37°C
($U/10^{12}$ RBC's) : 146 to 376 at 30°C / 202 to 522 at 37°C

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	**5 x 1 Test**	**5 x 5 Tests**
L1 : G6PDH Reagent	5 x 1 ml	5 x 5.5 ml
L2 : Starter Reagent	10 ml	50 ml

Storage / stability

Contents are stable at 2-8°C till the expiry date mentioned on the labels.

Reagent Preparation

Reconstitute G6PDH reagent (L1) with D.W. as per the volume mentioned on the label. This working reagent is stable for 6 hours at R.T. and at least 5 days when stored at 2-8°C.

The Starter Reagent (L2) is ready to use.

Sample material

Fresh whole blood sample collected in EDTA ,Heparin or ACD. Red Cell G6PDH in whole blood is reported to be stable for 7 days at 2-8 °C, but is unstable in hemolyzates. Freezing is not reccommended.

Procedure

Wavelength : 340 nm
Temperature : 30°C / 37°C
Light path : 1 cm

Pipette into clean dry test tubes Labelled Test (T)

Addition Sequence	**T** (ml)
G6PD Working Reagent (L1)	1.0
Whole Blood	0.01
Mix well & incubate for 5 -10 min. at R.T. and add	
Starter Reagent	2.0

Mix well & incubate for 5 min. at 30°C / 37°C and read the initial absorbance A_0 & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA/min.).

If the G6PDH activity is very low, the absorbance change per minute will also be very low. In such cases read the initial absorbance A_1 and read another absorbance A_2 exactly 5 min. later. Calculate the mean absorbance change per minute (ΔA/min.).

$$\Delta A/\text{min.} = \frac{A_2 - A_1}{5}$$

Calculations

$$\text{G6PDH Activity (U/10}^{12}\text{ RBC)} = \frac{47780 \times \Delta A}{\text{RBC Count in million}}$$

$$\text{G6PDH Activity (U/g Hb)} = \Delta A \times \frac{4778}{\text{Hb (g/dl)}}$$

TEMPERATURE CONVERSION FACTORS

Assay Temperature	**Desired Reporting Temperature**		
	25°C	**30°C**	**37°C**
25°C	1.00	1.32	1.82
30°C	0.76	1.00	1.39
37°C	0.55	0.72	1.00

Notes

Since the activity of G6PDH is reported in Hb. concentration or RBC count the same should be determined before performing the assay. RBCs are well preserved when collected in ACD and such samples give an accurate count , for samples collected in Heparin counts become unreliable after 2 days and in such cases results are best reported in Hb concentration.

Copper and Sulphate ions inhibit the G6PDH activity, hence use of good quality D.W for reconstitution of L1 and use of

properly cleaned glassware is essential.
Young red cells have a higher G6PD content then the older ones, regardless of the genetic variant that is present. If the enzymes have defective activity, older cells are preferentially destroyed during mild to moderate hemolytic phase. Since reticulocytes released to replace lost cells have high enzyme levels, falsely elevated results may occur if blood is tested immediately after a hemolytic episode.
Normallly the activity contributed by WBC, platelets or serum is very small. In cases of severe anemia, leucocytosis, or very low G6PDH levels, the use of a sample after removing the Buffy Coat is reccommended.

References

WHO, Tech. Rep. Ser No. 366, 1967.
Diagnostic Hematology by Rodak, W.B. Saunders, 1995 Ed. : 218.
Jacques Wallach, Interpretation of Diagnostic Tests, V Edition, page 315.
Tietz, Clinical Chemistry, Saunders (1986), page no, 1501-12.
Varley .H. Practical Clinical Biochemistry, V Edition, 729 - 713.

System Parameters

Reaction	:	Kinetic
Wavelength	:	340 nm
Zero Setting	:	Distilled Water
Incub. Temp.	:	37°C
Incub. Time	:	5 min. + 5 min.
Delay Time	:	30 sec.
Read Time	:	180 sec.
No. of read.	:	4
Interval	:	60 sec.
Sample Vol.	:	0.01 ml
Reagent Vol.	:	3.00 ml
Standard	:	---
Factor	:	47780/RBC Count 4778/Hb
React. Slope	:	Increasing
Linearity	:	---
Units	:	---

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