G6PDH

with Buffer

INTENDED USE

Vitro G6PDH reagent is a quantitative kinetic test for the determination of G6PD Activity in RBCs on both manual and automated systems.

METHOD

Kinetic method

CLINICAL SIGNIFICANCE

Glucose-6-Phosphate-Dehydrogenase (G6PD) deficiency is one of the most common human enzyme deficiencies 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 anemias 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.

ASSAY PRINCIPLE

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

$$\text{G 6 - P + NADP} \xrightarrow{\text{G6PD}} \text{Gluconate 6 - P + NADPH + H}$$

EXPECTED VALUES

7.9 –16.3 U/g Hb

202 – 522 U/10^{12} RBC's

Values for the newborns may range somewhat higher.

Since the normal values depend on age, sex, diet, geographic area and other factors, each laboratory should establish its own normal values for this procedure.

SPECIMEN

Whole blood sample collected in EDTA, Heparin or ACD. Red Cell G-6PDH in whole blood is stable for 7 days at 2-8 °C (Freezing is not recommended) and unstable in hemolysates. 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 the G-6PDH assay. The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts usually poses no problem. However, red cell counts on specimens collected in heparin become unreliable after about 2 days. Thus, for heparinized samples, results are best reported in terms of hemoglobin concentration.

REAGENTS

Buffer (R1)	Good buffer modified >20 mmol/L
NADP (R2)	NADP > 0.19 mmol/L
G6P (R3)	G6P > 0.1 g/L

- **Reagent Preparation & Stability**

Precautions

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R2) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: Dispose of this material and its container at hazardous or special waste collection point.

S57: Use appropriate container to avoid environmental contamination.

S61: Avoid release in environment.

For further information, refer to the G6PDH reagent material safety data sheet.

Storage and Stability

G6PDH working reagent is prepared by dissolving the content of the vial of R2 with 1mL of R1. Working solution is stable for 6 hours at room temperature and 5 days when stored at 2-8°C. Mix gently avoid foaming.

R3 is ready to use.

Let the reagent reach the room temperature before use. Close immediately after handling.

All reagents are stable until expiration date stated on label when properly stored refrigerated at 2-8°C.

Reagent deterioration: G6PDH reagent is normally clear, do not use reagent if it is turbid.

PROCEDURE

Wavelength	334 - 365 nm (340 nm)
Cuvette	1 cm light path
Temperature	37 °C
Zero adjustment	against distilled water
Specimen	Blood

	Specimen
Working Reagent	500 µl
Specimen	5 µl

Mix kindly and incubate for 10 minutes at *37 °C.*

R_3	1 ml

Mix well and incubate for 2 min. Read the initial absorbance, repeat absorbance reading after 5 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

For concentration of G6PDH higher than 3200 U/L use half sample volume and multiply the result x 2.

CALCULATION

G6PDH activity is expressed as U/L or U/g hemoglobin (Hb).

G6PDH (U/L) = ΔA/min x 48390

$$\text{G6PDH (U/g Hb)} = \frac{\text{G6PDH (U/L)}}{\text{Total Hb (g/dL) x 10}}$$

Where '10' is the multiplier that converts g/dl in g/l of total hemoglobin (Total Hb)

$$\text{G6PDH (U/10}^{12}\text{ RBC's)} = \frac{\text{G6PDH (U/L)}}{\text{RBC count in million}}$$

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Use of Buffy-Coat-Free Sample[4,5]

Under normal circumstances G6PD activity contributed by leukocytes, platelets and serum is relatively small. However, as reported by Echler and others, more accurate measurement of red cell G6PD activity, especially in the presence of anemia and/or leukocytosis, can be achieved by using buffy coat-free blood samples for assay. Thus, in case of a borderline value obtained with whole blood, it may be warranted to repeat the assay on a buffy coat-free sample.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established calcium values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

PERFORMANCE CHARACTERISTICS

Linearity limit: Up to 3200 U/L, under the described assay conditions.

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean IU/L	264	537	276	535
SD. IU/L	13.6	23.7	16	25.5
CV. %	5.2	4.4	5.8	4.8

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from ***VITRO*** G6PDH reagent compared with commercial reagent of the same methodology give a correlation of 0.990.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 27 U/L.

INTERFERING SUBSTANCES

Icterus
Bilirubin has negative interference above 7.5 mg/dL.

lipemia
Lipemic specimens interfere up to 4000 mg/dl.

Ascorbic acid
It interfere up to 50 mg/dl.

Others
Glucose, turbidity, copper and other drugs may interfere

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ORDERING INFORMATION

REF	SIZE
15501	10 TEST
15502	30 TEST

SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

Article # :155 -EN
Date of Revision : 03/2021

ISO 13485:2016 CE

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