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G6P-DH (UV/KINETIC)
QUANTITATIVE WITH CONTROLS

REF 038T

G6P-DH Determination With Washing of RBCs

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

UDI Diagnostics Glucose-6-phosphate; dehydrogenase reagents are for the quantitative, ultravoilet, kinetic determination of G6P-DH in blood at 340 nm. (EC 1.1.1.49; NADP 1-oxidoreductase) activity in erythrocytes (hemolysate),

DIAGNOSTIC SIGNIFICANCE

Most of the interest of G6P-DH focuses on its role in the erythrocyte. Here, it functions to maintain NADPH in its reduced form. An adequate concentration of NADPH is required to regenerate sulfhydryl-containing proteins such as glutathione form the oxidized to the reduced state. Glutathione in the reduced form, in turn, protects hemoglobin from oxidation by agents that may be present in the cell.

A deficiency of G6P-DH consequently results in an inadequate supply of NADPH and ultimately in the inability to maintain reduced glutathione levels. When erythrocytes are exposed to oxidizing agents, hemolysis occurs because of oxidation of hemoglobin and to damage of the cell membrane.

G6P-DH deficiency is an inherited Sex-linked trait. The disorder can result in several different clinical manifestations, one of which is drug-induced hemolytic anemia. When exposed to an oxidant drug such as primaquine, an antimalarial drug, affected individuals experience a hemolytic episode. G6P-DH deficiency is most common in blacks, but has been reported in virtually every ethnic group[1].

There is an evidence to suggest that the G6P-DH deficiency confers some protection against Falciparum malaria. Thus it may lessen the severity of malarial infections in young children and infants[2].

Full expression of trait occurs in hemizygous males, in whom the single X chromosome carries the mutant gene and in homozygous females in whom both sex chromosomes (XX) carry a mutant gene. Intermediate expression is found in heterozygous females, in whom expression is variable. Female heterozygotes have been shown to have two populations of red cells, one with normal and the other with markedly deficient enzyme activity. The relative proportion of the two populations present in different heterozygotes results in G6P-DH activities which may vary from almost normal to those found for hemizygotes[2].

A red cell hemolysate is used to assay for deficiency of the enzyme.

EXPECTED VALUES[3]

Following values were obtained from a frequency distribution of 150 "normal" individuals, of both sexes from Dammam Area in Eastern Provience, KSA

At 25°C	RBC:mU/10⁹ RBC	Hb: U/gHb
2.5th Percentile	118	4.07
95th percentile	141	4.97

METHOD PRINCIPLE

The enzyme G6P-DH (old name G6P-DH) catalyses the dehydrogenation of glucose 6-phosphate as the first step in pentose phosphate pathway. $NADP^+$, the electron acceptor, is reduced to NADPH in the reaction. The pH optimum for the G6P-DH reaction is 8.3 for the enzyme from yeast or blood cells. The rate of formation of NADPH is a measure of the G6P-DH activity and it can be followed by means of the increase in extinction at 340, 334 or 365 nm[4]. The overall reaction is outlined as follows:

$$\text{Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{G6P-DH}} \text{6-Phosphogluconate} + NADPH + H^+$$

UDI's G6P-DH procedure is referenced from Lohr and Waller[3] method and Bishop procedure [5, 6] for G6PD and PGD.

REAGENTS

1. ***G6P-DH BUFFER*** : Triethanolamine buffer 50 mmol/L, EDTA 5 mmol/L, pH 7.6 ± 0.05 (25 °C). Preservative added. Ready to use.
2. ***NADP REAGENT*** : (Concentration refer to reconstituted reagent) 30mM NADP.

RECONSTITUTION

Reconstitute each vial of NADP Reagent with the volume of distilled water indicated on the vial label. After the addition of distilled water, swirl gently to dissolve. Stable for 1 month when stored at 2-8 °C.

3. ***G6P-DH SUBSTRATE***: (Concentration refer to reconstituted reagent) Glucose-6-Phosphate Sodium Salt 17 mM. Preservative added.

RECONSTITUTION

Reconstitute each vial of G6P-DH SUBSTRATE Reagent with the volume of distilled water indicated on the vial label. After the addition of distilled water, swirl gently to dissolve. Stable for 1 month when stored at 2-8 °C.

4. ***G6P-DH LYSING REAGENT:*** An aqueous solution containing 0.2% Saponin. Ready to use.
5. ***G6P-DH DEFICIENT CONTROL : Ready to use lysate.***
   The lysate of this control contains 4.5x10 ^{9} RBC's per ml or 130 gHb/L of blood. The results obtained must be divided by 4.5 for RBC or 130 for Hb to get the expected value.

EXPECTED VALUE(At 25°C) :- RBC: 0 – 11 mU/10⁹ RBC.
Hb: 0.0 –0.39 U/gHb.

6. ***G6P-DH NORMAL CONTROL : Ready to use lysate.***
   The lysate of this control contains 4.5x10 ^{9} RBC's per ml or 130 gHb/L of blood. The results obtained must be divided by 4.5 for RBC or 130 for Hb to get the expected value.

EXPECTED VALUE(At 25°C) :- RBC: 80-180 mU/10⁹ RBC.,
Hb: 2.76 – 6.21 U/gHb.

STORAGE AND STABILITY

Store all reagents of this reagent set at 2 - 8 °C. All reagents are stable up to expiration date indicated on the individual bottle label prior to reconstitution. After reconstitution reagent 2 and reagent 3 are stable for 30 days when stored at 2 -8°C. The controls are stable upto the expiration date indicated on the label when stored at 2-8 °C.Once opened, the controls are stable for 30 days.

PRECAUTIONS

G6P-DH lysing reagent contains saponin which is harmful if taken internally. Avoid contact with eyes and skin. In case of contact flush with copious amount of water and seek medical attention. Exercise the normal precautions required for the handling of other reagents. Pipetting by mouth is not recommended for any laboratory reagent. The controls contain Sodium azide as preservative. Upon disposal, flush with large amounts of water to pervent azide build-up.

INDICATIONS OF REAGENT DETERIORATION

- Initial absorbance of reagent 1 and 2 mixture before addition of sample as more than 0.4 indicates reagent deterioration.
- Failure to obtain control values in the range may also indicate reagent deterioration.

NOTE : UDI cannot guarantee the stability of reagents which have been:

a) Transferred from their original containers.
b) Improperly stored.
c) Contaminated during use.

REAGENTS PROVIDED

G6P-DH Buffer, NADP Reagent, G6P-DH Substrate and G6P-DH Lysing Reagent, deficient control and normal control.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Saline (0.9% NaCl Solution), Test Tube Rack, Spectrophotometer with temperature controlled Cuvette/water bath, Centrifuge, Timer, etc.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Whole blood collected with ethylenediaminetetraacetic acid (EDTA), heparin or aicid-citrate-dextrose (ACD) is satisfactory.[3-7] Red cell G6P-DH is stable in whole blood for one week refrigerator (2-8^{0}C), but is unstable in red cell hemolysates.[8] freezing of bloods is not recommended.[3]

Since activity is reported in terms of number of red blood cells or grams hemoglobin, red cell count or hemoglobin concentration should be determined prior to performing the G6P-DH assay. The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts usually proses no problem.[5]. However, red cell counts on specimens collected in heparin become unreliable after about 2 days.[5] thus, for heparinized samples, results are best reported in terms of hemoglobin concentration. Once the hemolysate is prepared, the G6P-DH activity must be measured within 30 minutes.

INTERFERING SUBSTANCES

1. Copper completely inhibits G6P-DH at a concentration of 100 umol/L, and sulfate ions (0.005 mol/L) decrease observed levels of G6P-DH activity[8].
2. Certain drugs and other substances are known to influence circulation level of G6P-DH[9].
3. Reticulocytes have higher G6P-DH levels than mature red cell. It is recommended that assays not be performed after a severe hemolytic crisis, since G6P-DH levels may appear falsely elevated. Under those conditions, detection of deficiency may require family studies. Testing may be performed after the level of mature red cells has to returned to normal.
4. Under normal cirumstamces, activity contributed by leukocytes, platelets and serum is relatively small. However, in cases of extreme anaemia,grossly elevated white counts or, very low levels of red cell G6P-DH activity, the contribution to the total made under these conditions may be significant.

PREPARATION OF HEMOLYSATE

1. Count the number of erythrocytes per ml of blood.
2. Wash 0.2 ml of blood three times with 2 ml aliquots of saline (0.9% Nacl). Centrifuge after each washing for 10 minutes at approx 3000 rpm. Remove the saline layer completely without any loss of erythrocytes.
3. Resuspend the erythrocytes in 0.5 ml of reagent 4 (G6P-DH lysing reagent) and allow to stand for 10 minutes at room temperature. Centrifuge immediately for 3 minutes at 3000 r.p.m. Use 50 µl of supernatent hemolysate as a sample for the erythrocytes activity determination.

PROCEDURE PARAMETERS

Wavelength .....340 nm.
Reaction Type .....Delta Kinetics with factor.
Units ....mU/Erythrocytes per ml of blood* U/L or mU/ml
Factor 30868
Incubation Time .....30 seconds.
Interval Time .....60 Seconds.
Number of Intervals .....3.
Temperature .....25°C.
Reaction Slope .....Increasing.
(*See calculations)

PROCEDURE (MANUAL)

Pipette into clean and dry test tubes:

G6P-DH Buffer **(Rgt. 1)** NADP Reagent **(Rgt. 2)** Hemolysate (Supernatent) /Controls	3.0 ml 100 µl 50 µl --
Mix and incubate for 5 minutes at 25 °C, then add:	
G6P-DH Substrate **(Rgt. 3)**	50 µl
Mix and transfer to cuvette at 25 °C. After 30 seconds read initial absorbance at 340 nm against distilled water. Repeat absorbance readings every minute for the next 3 minutes and calculate the mean Δ A/min.	

CALCULATIONS

Δ A/min x 30868 = G6P-DH Activity in **mU/erythrocytes per ml of blood = P.**

If the erythrocytes count per ml of blood is 5 x 10^9, then the G6P-DH activity in mU/10^9 cells = $\frac{P}{5}$

UNIT DEFINITION

In Unit of enzyme activity is the amount of enzyme that will convert 1µmol of glucose-6-phosphate per minute to 6-phosphogluconate under the specified conditions of reaction.

CALCULATION OF FACTOR

Following equation has been used to derive calculation factors.

G6P-DH activity in mU/erythrocytes per ml of blood.

$$= \frac{\Delta \text{ A/min}}{\varepsilon} \times \frac{TV}{SV} \times \text{Conv. Factor} \times \text{Dil. Factor.}$$

Where:
- Δ A/min. = Measure of rate of change of absorbance per minute (Mean) at 340 nm.
- ε = Millimolar absorptivity of NADP at 340 nm = 6.22 L X $mmol^{-1}$ X cm^{-1}.
- TV = Total volume of reaction mixture.
- SV = Sample Volume.
- Conv. Factor = 1000 (Factor to convert millimole to micromoles).
- *Dil. Factor = 3 (applicable to erythrocytes only).

Therefore for erythrocytes, G6P-DH activity in mU/erythrocytes per ml of blood = $\frac{\Delta \text{ A/min.}}{6.22} \times \frac{3.2}{0.05} \times 1000 \times 3^* = \mathbf{10289.4 \times 3^* = 30868}$

* This dilution factor is calculated based on the PCV of 0.45 (1/1) and smallest possible dilution of packed erythrocytes with saline (approximately 0.010 ml), i.e. 0.2 ml of whole blood after 3 times washing and centrifuging of the saline will contain 90 µl (45%) cells and approx. 10 µl of saline. Therefore, the dilution factor after adding 0.5 ml of lysing reagent to packed cells will be:

$$\frac{0.5 + 0.090 + 0.010}{0.200} = 3$$

NOTE : Samples from anemic persons will have less PCV. Therefore, appropriate correction to be made as follows, while calculating the factor:

$$\frac{0.5 + (0.2)\,PCV + 0.010}{0.2}$$

QUALITY CONTROL

Reliability of test results should be monitored by use of Normal and Deficient controls within each run. Udi glucose-6 phosphatase dehydroganase (G6P-DH) controls are suitable for this purpose. A control range should be established by the laboratory to determine the allowable variation in day to day performance of each control.

Controls falling outside the upper or lower limit of the established ranges indicate the assay may be out of control. Failure to meet quality control specification should be investigated and resolved. Its suggested whenever controls are not within the established acceptable range.The assay should then be repeated if problem cannot be resolved contact Udi technical service.

PERFORMANCE CHARACTERISTICS

ASSAY RANGE: The maximum G6P-DH activity which may be measured by this procedure is approximately 24.6 U/gHb or 712 mU/10^9 RBC.

PRECISION: Precision studies were performed on a udi semi automated analyzers folowing the guidelines contained in NCCLS document EP5-T2[10].

Normal Control Within run (N=20)				Normal Control run to run (N= 20)			
	Mean	SD	CV %		Mean	SD	CV %
RBC	254.3	4.88	1.92	RBC	231.6	8.03	3.47
HB	8.80	0.17	1.92	HB	8.02	0.28	3.47

Range: RBC: 135-277 mU/10^9 RBC., Hb: 4.66-9.55 U/gHb

NOTE: The obtained coefficient of variation (CV) for normal controls is acceptable in below conditions:

1.Less than 5% for within run assay value.

2.Less than 10% for run to run assay value.

Deficient Control Within run (N=20)				Deficient Control run to run (N = 20)			
	Mean	SD	CV %		Mean	SD	CV %
RBC	2.86	2.21	77.3	RBC	1.24	0.87	70.2
HB	0.10	0.08	77.3	HB	0.04	0.03	70.2

NOTE:

1.Coefficient of Variation (CV) also known as relative variability or measure of variation. It gives nonsensical results for deficient controls as the values are negative or zero. A weight of zero means no weight .An enzyme activity of zero means no enzyme activity.Therefore it makes no sense to compute the CV of deficient values. In such cases Udi recommends to compare measures of actual variability like :

Range: RBC: 0-11 mU/10^9 RBC., Hb: 0-0.39 U/gHb

2.Or else to add a constant value 50 to each obtained deficient value and then calculate the Coefficient of Variation (CV)as adding a constant will change the CV but not change the variation.

https://www.graphpad.com › quickcalcs › ttest

https://stats.stackexchange.com › questions › why-is-the-coefficient-of-vari.

3. In deficient control the zero "0" disadvantage. CV is useful only for the calculations, when the mean of sample is not zero lets assume: If the sample mean is equal to zero than the denominator would became zero hence the CV gets nullified.

SENSITIVITY: Assuming the limit of sensitivity to represent a change in absorbance at 340nm of 0.001 per minute a G6P-DH activity of 0.4 U/g Hb or 11 mU/10^9RBC.may be detected using this procedure (assuming a hemoglobin concentration of 130 g/L and a red cell of 4.5 X mU/10^9 RBC.

SPECIFICITY: The oxidation of glucose-6- phosphate by G6P-DH is specific. Any non-specific formation of NADPH due to oxidation of other substrates by endogenous enzymes occurs during the preincubation period 6-Phosphogluconate dehydrogenase is completely inhibited by maleimide in the reagent system.

CORRELATION : UDI reagent tested on MANUAL SYSTEMS (y) was compared with another commercial reagent (x). The systematic difference between the results were within CLIA specified limits. N=18.

Correlation Coefficient	0.97
Regression Equation	y = 0.83x + 0.39

PRECISION: (Within run)

	Nos samples	Mean U/L	SD	CV%
Samples	21	254.3	4.76	1.87

PROCEDURE NOTES

1. If Δ A/min is found to be more than 0.060 dilute the specimen (Hemolysate) 1:10 with saline (i.e. 0.1 ml specimen + 0.9 ml saline) and repeat the assay. Multiply the results by 10.
2. In case, G6P-DH activity is found to be very low, continue the absorbance readings every minute for a total of 5 minutes and calculate Δ A/min.
3. If the initial absorbance is found to be more than 1.8, blank the instrument with 3.05 ml of buffer + 0.05 ml sample and read against this blank.
4. The controls contain Sodium azide as preservative. Upon disposal, flush with large amount of water.
5. The deficient control, at times may give negative Δ A/min. Consider the value as Zero.
6. The relative activity of G6P-DH at several selected temperatures are: 25 °C, 0.72; 28 °C, 0.89; 30 °C, 1.00; 32 °C, 1.12; 35 °C, 1.33; 37 °C, 1.52[5].
7. Source of error:
   G6P-DH is inhibited by primaquine[7] and other 8- aminoquinolines (antimalarial drugs) in millimolar concentration as well as by phenyl hydrazine. Nevertheless, the therapeutic concentration of these substances is more than ten fold lower and therefore, they have no significant effect on the measurement.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For Technical assistance, please call 00966 13 8121217.
For sales call 00966 13 8200016.

PRODUCT AVAILABILITY

G6P-DH REAGENT SET (UV/KINETIC)

REF # 038T-020 1 x 60 ml
REF # 038T-040 2 x 60 ml

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Langenhagen

EC REP
Authorized representative in the European community)

CE

G6P-DH DETERMINATION REF 038T

G6P-DH Determination Without Washing of RBCs

An alternate method for the determination of G6P-DH may be followed. In this method, there is no necessity to prewash the blood samples with saline.

MANUAL METHOD

Pipette 400 micro litres of lysing reagent to a test tube. Add 100 micro liters of well mixed whole blood. Mix well and wait for 5 minutes.

In another test tube pipette 3 ml of buffer and add 100 micro litres of NADP reagent. To this mixture add 50 micro liters of the lysate prepared in step I. Wait for further 5 minutes.

To the above mixture add 50 micro litres of the substrate and take the absorbance exactly after 1 minute (A1). After exactly 5 minutes later take the second absorbance reading(A2).

By any standard procedure determine the RBC concentration (millions) of the given whole blood sample.

Calculation:

$$\text{Delta absorbance} \quad \frac{(A2 - A1) \quad X \ 51447}{\text{RBC mU}/10^9 \quad \text{value x } 5}$$

SEMI AUTOMATED METHOD

A semi auto analyser may be set to to the following Program. The final reaction mixture after the addition of substrate may be directly fed to the instrument to get the results in mU/ml. This result should be divided by the RBC value (millions) of the sample to get the G6P-DH milli Units / RBC (millions)

Program for the semi auto analyser: Delta Kinetics With Factor

Units:	mU/ml
Filter:	340nm
Factor:	51447
Incubation Time:	60 Secs
Interval Time	60 Secs
Number of Intervals	5
Temperature	25 ^{0}C
Sipping volume	1000 µl
Reaction Slope	Increasing

EXPECTED VALUES FOR DEFICIENT CONTROL:- RBC: 0 - 11 mU/10^9 RBC
Hb : 0 – 0.39 U/gHb

EXPECTED VALUE FOR NORMAL CONTROL :- RBC 135 – 277 mU/10^9 RBC
Hb 4.66 – 9.55 U/gHb.

EXPECTED VALUES FOR PATIENTS :-

At 25°C	RBC:mU/10^9 RBC	Hb: U/gHb
2.5th Percentile	100	3.36
95th percentile	248	8.60

Health and Safety Information

1. It is recommended to handle the reagents carefully, to avoid ingestion and contact with eyes, skin and mucous membranes and to use the reagent according to good laboratory practices. Do not pipette by mouth. Wear disposable gloves and eye protectors while handling reagents and performing the assay. Wash hands thoroughly when finished.

2. The reagent contains low concentrations of harmful and/or irritant substances as preservatives.

3. **Majority of reagents contain Sodium Azide that can react with lead and copper in plumbing, forming highly explosive deposits of metal azides; dilute with large amount of water to eliminate any possible deposits**

4. Non-disposable apparatus should be sterilized after use .The preferred method is to autoclave for one hour at 121°C; disposables should be autoclaved or incinerated. (or as per the local environmental management system regulations)

5. Neutralized acids and other liquid waste should be decontaminated by adding sufficient volume of hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to one percentage sodium hypochlorite may be necessary to ensure effective decontamination.

6. Spilling of potentially infectious material should be removed immediately with, absorbent paper, tissue and the contaminated area swabbed with. Material used to clean spills, including gloves, should be disposed of as potentially biohazardous waste. Do not autoclave the material containing sodium hypochlorite.

IVD	For in vitro Diagnostic use
REF	Catalogue Number
LOT	Batch code
	Storage Temperature
	Date of manufacture
	Use by
	Instructions For use
	Important Notice
CONT	Contents
	Manufacturer
	Contains sufficient for <n> tests
	This product fulfils the requirements of the European directive 98/79 EC for in vitro diagnostic medical devices.
	TOXIC
Xn 	Harmful
EC REP Authorized European representative	European Community representative
	Biological risk
Xi 	Irritant