CE IVD DIALAB

Dry Powder Reagents

G6PDH Deficiency Screen
(Glucose-6-Phosphate Dehydrogenase)

qualitative, visual
2 Reagents

Diagnostic reagent for qualitative in vitro determination of Glucose-6-phosphate dehydrogenase deficiency in human red blood cells

REF Cont.

Y04400	**12 x 10 ml**	12 x 10 ml	Reagent 1
		2 x 60 ml	G6PDH Buffer

Additionally offered:
Y04560 6 x 0.5 ml G6PDH Control Set

TEST PARAMETERS

Method:	Visual, colorimetric
Wavelength:	- -
Temperature:	37°C
Sample:	Whole blood with EDTA, heparin or acid-citrate-dextrose (ACD)
Linearity:	- -
Sensitivity:	- -

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent 1:		
NADP	0.5	mM
Glucose-6-phosphate	4.55	mM
Dichlorophenol indophenol	0.55	mM
Phenazine methosulfate	0.2	g/L
Buffer:		
Buffer to give pH of 8.5 ± 0.1 when reconstituted with Reagent 1		
Sodium azide	0.095	%

REAGENT PREPARATION

Reagent 1: Reconstitute the content of each vial with the volume of G6PDH buffer indicated on the label. Swirl gently and invert several times to dissolve contents. Wait 2-3 minutes and mix again.
G6PDH Buffer: The buffer is ready to use.

REAGENT STABILITY AND STORAGE

Conditions: protect from light
Storage: at 2 – 8°C
Stability: up to the expiration date

After reconstitution: at 2-8°C 24 hours

SAMPLE PREPARATION

Prepare a red blood cell hemolysate by adding 0.05 ml (50 µl) whole blood to 2.5 ml deionized water. Mix gently and allow to stand for 5 minutes.

SAMPLE STABILITY AND STORAGE

Whole blood:
Stability: at 2 – 8 °C 7 days

Hemolysate: unstable

Do not freeze! Discard contaminated specimens.

INTERFERING SUBSTANCES

no interference up to:

copper	100 µmol/L
sulphate ions	0.005 mol/L

Certain drugs and other substances are known to influence circulation levels of G6PDH.[11]
Reticulocytes have higher G6PDH levels than mature red cells. It is recommended that assays not be performed after a severe haemolytic crisis, since G6PDH 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.
Under normal circumstances, activity contributed by leukocytes, platelets and serum is relatively small. However, in cases of extreme anemia, grossly elevated white counts or, very low levels of red cell G6PDH activity, the contribution to the total made under these conditions may be significant.

MANUAL TEST PROCEDURE

Pipette into a test tube:	
Reagent	500 µl
Hemolysate	1000 µl
Gently shake tube to mix. Tightly close the tube with a rubber stopper or sealing film (e.g. Parafilm®) or gently layer approximately 1-2 ml of mineral oil on top of the reaction mixture to avoid evaporation. **Do not mix the mineral oil with the reaction mixture!** Place the tubes into a 37°C heating block or water bath.	
Observe the tubes at 15 minute intervals for up to 1 hour looking for a colour change from the original deep blue/purple to a red/orange endpoint. The endpoint may be more easily detected if the tubes are viewed in front of a bright light or white paper	

RESULTS

Normal blood (normal levels of G6PDH) will typically reach the red/orange endpoint within 15-60 minutes.

REFERENCE RANGE

Samples were collected from 152 apparently healthy adults and assayed according to this method. Every sample reached the red/orange endpoint within 60 minutes.
This assay is designed to detect samples with a significantly deficient level of G6PDH from those with an essentially normal level of G6PDH. It is strongly recommended that any samples requiring longer then 60 minutes to reach the red/orange endpoint be assayed using a quantitative G6PDH method to verify the finding of deficiency.

TEST PRINCIPLE

Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) and reducing NADP to NADPH:

$$G\text{-}6\text{-}P + NADP^+ + \xrightarrow{G6PDH} 6\text{-}PG + NADPH + H^+$$

In the presence of phenazine methosulfate (PMS), NADPH reduces dichlorophenol indophenol from its blue form to its colorless form. The rate at which the blue color disappears is dependant on the G6PDH present in the red cells.

PERFORMANCE CHARACTERISTICS

PRECISION

A known normal sample and a known deficient sample were run in duplicate on the successive days. The known normal sample was found to be normal in 100 % of the assays. The known deficient sample was found to be deficient in 100 % of the assays.

METHOD COMPARISON

A comparison study on 164 samples between the Dialab method and that of Sigma Diagnostics yielded 100% agreement in the results from the two methods. The Samples included 157 normal specimens and 7 deficient specimens.

This visual colorimetric procedure has also been verified in comparisons with the methemoglobin reduction method and the ascorbate-cyanide method.[12]

QUALITY CONTROL

Reliability of test results should be monitored by use of control materials with known lwvels of G6PDH within each run.
We recommend:

REF Cont.

Y04560 6 x 0.5 ml G6PDH Control Set

WARNINGS AND PRECAUTIONS

1. These reagents are for in vitro diagnostic use only.
2. Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.
3. R1 Reagent is toxic. May cause genetic damage and/or irritation to eyes, respiratory system and skin. Wear suitable protective clothing.
4. G6PDH Buffer contains sodium azide that may react with lead and copper plumbing to form highly explosive metal azides. Avoid azide accumulation by flushing with copious amounts of water upon disposal.

WASTE MANAGEMENT

Please refer to local legal requirements

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