I. PRINCIPLE:

NAPD is reduced by glucose-6-phosphate dehydrogenase in the presence of Glucose-6-Phosphate as follows:

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

The rate of formation of NADPH is proportional to the G-6-PDH activity, and may be determined spectrophotometrically as an increase in absorbance at 340 mm. Production of a second molar equivalent of NADPH by 6-phospho-gluconate dehydrogenase, also present in erythrocytes according to the reaction:

$$\text{6-PG + } NADP^+ \xrightarrow{\text{6-PGDH}} \text{Ribulose-5-Phosphate + NADPH + } H^+ + CO_2$$

is prevented by the presence of maleimide which inhibits the 6-PGDH.

II. SPECIMEN:

EDTA (lavender vacutainer) stored in the refrigerator (2-8^{o}C). G-6-PDH is stable in the intact erythrocyte for 7 days when refrigerated. Since activity is reported in terms of number of grams of hemoglobin, the hemoglobin concentration should be determined prior to performing the G-6-PDH assay. **Also check the WBC and platelet counts,** if elevated a buffy coat free sample may need to be used.

Prepare Buffy Coat-Free Samples if any of the following apply:

In cases of extreme anemia (3 gm Hgb)
Grossly elevated white counts (>50,000, a leukemia patient)
Elevated platelet counts (1.000.000+)

In these cases the contribution of non red cells to total activity made be significant.

- To obtain a buffy coat-free sample: spin the blood at 3000 rpm for 10 minutes and extract the plasma/buffy layer with a glass 5 ¾ inch pipette.

Reject samples that are clotted.

It is not recommended that assays be performed after a severe hemolytic crisis, since G-6-PDH levels may appear falsely elevated since reticulocytes have higher levels than mature red cells. Under those conditions, detection of deficiency may require family studies. Testing may be more helpful after the level of mature red cells has returned to normal.

III. REAGENTS: Use only reagents that are included in the kit (assay vials and substrate). Do not mix reagents of different kits.

A. G-6-PDH Reagent Five-assay vial Stock No. 345-5
Reconstituted reagent will contain NADP 1.5µ mol and Maleimide 12 µ mol. Vials also contain buffer, stabilizer and lysing reagent. Store dry vials in refrigerator. Reconstituted reagent stable for 8 hours @ room temp.

CAUTION: Maleimide is an irritant. Avoid ingestion, inhalation, or contact with eyes or skin.

B. G-6PDH Substrate Solution Stock No. 345-8
Contains glucose-6-phosphate, 1.05 mmol/L, buffer and magnesium salt.
Also contains 0.1% Sodium Azide as a preservative. Store in refrigerator. Stable until expiration date shown on the label.

IV. CONTROLS: Do not mix lots of controls. Notify a supervisor when a new yearly lot of G6PD controls are delivered.

Reconstituted controls are stable for 1 week at 0 to -20 degrees.

A. Normal G-6-PDH
Catalog No.1 G6888
Lyophilized control containing a normal level of
G-6-PDH in a stabilized human red cell hemolysate base.
Preparation: Add 0.5 ml of distilled water, let the solution stand for 5 minutes and swirl until dissolved.

B. Deficient G-6-PDH
Catalog No. G5888
Lyophilized control containing a deficient level of
G-6-PDH in a stabilized human red cell hemolysate base.
Preparation: Add 0.5 ml of distilled water, let the controls stand for 5 minutes and swirl until dissolved.

C. Intermediate G-6-PDH
Catalog No. G5029
Lyophilized control containing an intermediate level of
G-6-PDH in a stabilized human red cell hemolysate base.

Preparation: Add 0.5 ml of distilled water, let the controls stand for 5 minutes and swirl until dissolved.

V. PROCEDURE:

A. To one vial G-6PDH reagent vial add: **5.5 ml distilled water**.
Swirl gently and invert several times to dissolve contents. Wait 2-3 minutes and mix again. Dispose of extra reagent when the test is completed. Each vial allows for 5 assays, reconstitute sufficient reagent for number of samples, controls and possible repeat assays.

B. Turn on the spectrophotometer to 340 nm. Blank to 0.0 with distilled water. Check the water bath. The temperature should read 37°.

C. Obtain Hgb concentration, WBC and Platelet count of patient sample by running it through the main hematology analyzer if not previously done. Process accordingly if sample requires buffy free preparation.

D. To pre-labeled 12x75 test tubes add **1.0 ml** reconstituted G-6PDH reagent.

E. Mix and check sample for clot. Add 10 μL of patient or control sample to the 12 X 75 tube. Cap and mix thoroughly to completely suspend erythrocytes. Let stand **10 minutes** at room temperature.

F. Add 2.0 ml of G6PDH substrate solution to control or patient tube, mix gently by inverting several times.

G. Immediately place in 37 ° water bath for 5 minutes to obtain thermal equilibrium

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H. Exactly 5 minutes later, read the INITIAL absorbance then return the specimen to the water bath.

I. Exactly 5 minutes later, again read and record absorbance. This is the FINAL absorbance.

J. To determine G-6-PDH activity, refer to "Calculations" section. **Use the Excel worksheet to determine correct/reportable result.**

VI. CALCULATIONS:

$$\text{G-6-PDH (U/g Hb)} = \frac{\text{Final A- Initial A}}{5} \times \frac{4839}{\text{Hgb(g/dL)}} \times 0.66 \text{ (temp correction for 37°)}$$

If Final A-Initial A/5 is greater than 0.060, repeat determination using 5 ul blood and multiply results by 2

Computer computation: (((B4-A4)/5*.66*(4839/C4))
Click on Start (lower left corner)
Click on Program
Access Microsoft Excel
Click on File (upper left corner)
Click on Open
Should be under "My documents"
Click on G6PD Cal
Type in the OD's and Hgb values
Click on the #DIV/o! to give you the final calculated answer
Enter new values over old values and repeat above procedure
Click on File
Click on Exit
Do save results. **(Y)**

VII. G6PD QC Instructions:

Results for controls are verified before entering patient results.
Enter all control values (in or out of range) into the Soft computer program. The Soft program will notify you if the results are not within range then comment in the SOFT QC (action). **Do not send out patient results. Document repeats Initial and Final absorbance values on the work sheet for both patient and control samples.**

- Click on Total QC Live Icon
- Click on Orders
- Click on Order Entry
- Click on 
- Item Type: Control
- Item ID: G6PDD (Deficient)
- Click on Find
- Click 
- Click Yes to "Do you want to save changes?"

- Order Entry will bridge to Result Entry
- Enter results
- If result is out of range choose Action from Action ID then Click OK
- Click Verify All
- Click 
- Click Yes to "Do you want to save changes?"

- Click on 
- Item Type: Control
- Item ID: G6PDI (Intermediate)
- Click on Find
- Click 
- Click Yes to "Do you want to save changes?"
- Order Entry will bridge to Result Entry
- Enter results
- If result is out of range choose Action from Action ID then Click OK
- Click Verify All
- Click 
- Click Yes to "Do you want to save changes?"

- Click on 
- Item Type: Control
- Item ID: G6PDN (Normal)
- Click on Find
- Click 
- Click Yes to "Do you want to save changes?"
- Order Entry will bridge to Result Entry
- Enter results
- If result is out of range choose Action from Action ID then Click OK
- Click Verify All
- Click 
- Click Yes to "Do you want to save changes?"

VIII. RESULTS:

A result of **<2.0 is a panic value** and needs to be communicated to the doctor in charge of the patient.

Normal Range:
Adults (mixed population) Normal (7-11 IU / gm hgb)
Borderline (4-6 IU / gm hgb)
Deficient (0-3 IU / gm hgb)

IX. INTERFERING SUBSTANCES:

Copper, which completely inhibits the enzyme at a concentration of 100 μ mol/L, and sulfate ions (0.005 mol/L) will decrease observed levels of G-6-PDH activity. Certain drugs and other substances are known to influence circulating levels of G-6-PDH.
Reticulocytes have higher G-6-PDH levels than mature red cells. Therefore, it is not recommended that assays be performed after a severe hemolytic crisis, since G-6-PDH levels may appear falsely elevated. Under those conditions, detection of deficiency may require family studies. Testing may be more helpful after the level of mature red cells has returned to normal.

X. REFERENCE:

Package insert, "Glucose-6-Phosphate Dehydrogenase," Procedure #345-UV, Sigma Diagnostics, St. Louis, Mo.

XI HISTORY:

H-1 This procedure was written by Paula Morris on 09/07/99.
H-2 This procedure was revised by Paula Morris on 12/06/10.
H-3 This procedure was revised by Paula Morris on 5/5/11.
H-4 This procedure was revised by Susan Richardson on 6/20/12.
H-5 This procedure was revised by Andrew Link on 6/27/2013