Title: G-6-PDH Screen –Trinity Biotech
SH.CP.AU.hem.0040.0003

Author (original):	Effective Date (original): *Note: The Effective Date is assigned after all approval signatures are obtained*	Supersedes Procedure #
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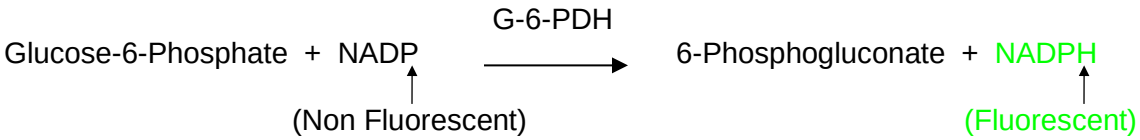
REVISION HISTORY

Document #	Revision Date	Reason for Revision
SH.CP.AU.hem.0040.0002		Version retired when kit was no longer available – 6/19/2017
SH.CP.AU.hem.0040.0003	**3/8/2018**	Procedure reintroduced due to shortage of current Binax NOW kits; updated to include new Deficient QC material, updated format

Title: G-6-PDH Deficiency Screening Test - Trinity Biotech

I. PURPOSE

The Sigma procedure is a modification of the Beutler method, involving the reaction:



G-6-PDH deficiency in red cells has been demonstrated to be the basis for certain drug-induced hemolytic anemias. The majority of subjects who have demonstrated G-6-PDH deficiency are clinically normal until exposed to one of several oxidant drugs (anti-malarial drugs, sulfa drugs, ascorbic acid, and others). This defect should be considered whenever an otherwise unexplained case of hemolytic anemia is encountered. The hemolytic episode is usually self-limiting. Young cells that are produced in response to the anemia have levels of G6PD that are nearly normal.

G-6-PDH deficiency is transmitted by a gene located on the X chromosome. The disorder is fully expressed in men, XY. In women, full expression of the disorder occurs only when two mutant genes, XX are inherited. Heterozygous women have two populations of red blood cells. One population has normal enzyme activity and the other has deficient enzyme activity.

Red cell G-6-PDH deficiency has been found in about 13% of African-American males and in about 3% of African-American females. The incidence is also high among other racial and ethnic groups, such as Sardinians, Greeks, and Sephardic Jews.

The test is performed by incubating a small amount of blood with glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate [NADP] at 37°C. Drops of the mixture are removed at 0, 5, and 10 minute intervals, spotted on filter paper, and then viewed under long-wave ultraviolet light. Fluorescence is clearly evident in mixtures prepared from normal blood, whereas deficient samples yield little or no fluorescence.

Deficient/ inconclusive samples are sent to the reference lab for confirmatory/ quantitative testing.

II. SCOPE

This procedure will be used by staff of the UR Medicine Hematology-Chemistry Laboratory for the detection of G-6-PDH deficiency.

III. RESPONSIBILITIES

Roles	Responsibilities
Quality	Ensure that procedure is followed when performing the G-6-PDH screening test.
Medical Director	Approve the G-6-PDH screening test procedure.
Management	Ensure that procedure is followed when performing

	the G-6-PDH screening test.
Technologists	Follow procedure.

IV. ACRONYMS

UR	University of Rochester
SMH	Strong Memorial Hospital
G6PDH	Glucose-6-Phosphate Dehydrogenase
NADP	Nicotinamide Adenine Dinucleotide Phosphate

V. SPECIMENS

A. **Specimen:** Venous blood collected by standard venipuncture procedure, into 1 EDTA tube. Minimum volume is 500µl, microtainer is acceptable. If blood is refrigerated, allow it to come to room temperature (64°F to 77°F) and mix well prior to testing.

B. **Specimen Storage:** Samples are stable at room temp (64°F to 77°F) for 8 hours and up to one week refrigerated (2-8°C). **Do not freeze samples prior to testing.**

C. **Handling Precautions:** Patient samples, controls, and test devices, should be handled as though they could transmit disease. Observe established infection prevention precautions.

VI. QUALITY CONTROL

A. Normal control: Trinity Biotech G-6-PDH (catalog # G6888) a lyophilized human red cell hemolysate containing a normal level of G-6-PDH, stored in refrigerator at 2-8°C. Once reconstituted, the reagent is stable for two weeks when stored at -20°C. The control is treated in the same manner as a patient sample.

B. Deficient control: Trinity Biotech G-6-PDH (catalog # G5888) a lyophilized human red cell hemolysate containing a deficient level of G-6-PDH, stored in refrigerator at 2-8°C. Once reconstituted, the reagent is stable for two weeks when stored at -20°C. The control is treated in the same manner as a patient sample.

C. Controls will be run once per day, prior to patient testing, when there are patient samples to be tested. Control materials give comparative levels of fluorescence for estimating whether the test specimen is normal or G6PDH deficient.

VII. SPECIAL SAFETY PRECAUTIONS

A. The Standard Universal Precautions recommended by the Centers for Disease Control should be followed whenever blood or body fluids are handled. These precautions include wearing gloves (and other personal protective equipment, if appropriate).

B. Dispose of collection equipment and specimens in a proper biohazard waste according to SMH Infection Control Policies.

C. Always use puncture resistant containers for sharps such as needles and biohazard bags/boxes for non-sharps.

D. Refer to Safety Data Sheets in SDS Manual for safety information.

VIII. MATERIALS

A. Equipment

1. Long-wave ultraviolet lamp
2. 37°C Water bath
3. Heat gun

B. Supplies

1. Trinity Biotech G-6-PDH Deficiency test kit (Catalog # 203-A)
    a. Trizma Buffer (Catalog # 203-2A)
    b. G-6-PDH Substrate (Catalog # 203-2B)
    c. Stored at 2-8°C unprepared
    d. Reconstituted Substrate is stable 2 weeks stored at -20°C.
2. Automatic pipettes that reliably deliver 10 µl, 200 µl, and 2.0 ml.
3. 20 µl micropipets
4. Filter paper - Fisherbrand Catalog # 09-802-1A. Must be cut into cards 7.5 x 9.5cm prior to testing.
5. Calibrated Timer.
6. 10x75 Disposable glass culture tubes
7. 2- Large wood clamps

C. Reagents

1. Distilled water
2. Saline: 0.9% Sodium Chloride, Irrigation grade

IX. PROCEDURE

Precautions:

- Do not mix components from different kit lots or shipments.
- Allow all samples, test devices, sample preparation tubes, and reagents to equilibrate to room temperature before use.

A. Testing Procedure

1. Check sample for clot. Partially clotted samples may be used as long as you have enough red cells to adjust hematocrit to between 30-35%. Fully clotted samples are not acceptable for testing.

2. Test the sample on the hematology analyzer to determine hematocrit and reticulocyte count. Record the results as internal tests in soft. See the LIMITATIONS section to confirm sample is acceptable for testing. If Retic count is ≥5%, add comment @SG6R.

3. If necessary, adjust the hematocrit to 30-35% by either removing plasma to raise the hematocrit or adding normal saline to lower the hematocrit. Refer to SH.CP.AU.jad.151.001 HCT Adjustment Table.

4. Prepare 1 precut filter card as shown below:

0 min	5 min	10 min	Patient ID	Result
			Normal Control	
			Deficient Control	
			Add one row for	
			each patient	

5. Place card in wood clamps to suspend it above the work surface.

6. Place one 10 x 75 tube for normal control, deficient control, and each patient in the water bath set at 37°C.

7. Add 200 µl of substrate to each tube.

8. Pipette 10 µl of normal control to the corresponding tube, mix by swirling, and promptly spot the mixture to the filter paper identified as “0 min - Normal Control.” Return sample to water bath.

9. Start timer counting up

10. Repeat step 8 with the deficient control and each patient, spotting in the corresponding positions in the graph. Note at what time each sample was added to the substrate (typically 30-45 seconds between samples).

11. When the timer reads 5:00 minutes, spot the normal control –substrate mixture to the square: 5 min - Normal Control.

12. Monitor the timer and spot the remaining samples at the appropriate 5 minute delay from their preparation, to their corresponding positions in the “5 min” column.

13. When the timer reads 10:00 minutes, spot the normal control–substrate mixture to the square: “10 min- Normal Control”.

14. Monitor the timer and spot the remaining samples at the appropriate 10 minute delay from their preparation, to their corresponding positions in the “10 min” column.

15. Dry the filter paper using the heat gun.

Note: Moisture decreases fluorescence so dry filter paper as soon as possible.

16. Visually inspect the dried spots on the filter paper using the long wave UV light in a dark room. Refer to INTERPRETATION section.

17. Test cards are stored in refrigerator for one month then discarded.

X. LIMITATIONS

A. A reticulocyte count of greater than 5.0% may cause a false normal reading. Young red blood cells (reticulocytes) have higher G-6-PDH levels than mature erythrocytes. A false normal result may be obtained in individuals with anemia and resulting reticulocytosis. It is important to remember to enter the comment "Elevated retic may cause a false normal G-6-PDH screen. Suggest repeat screen when the retic count is normal". In the comment section enter @**SG6R** followed by F5.

B. Donor RBCs can mask a G6PD deficiency. Search each patient's record for recent transfusions.

1. If the patient has been transfused RBCs < 30 days prior to sample collection, .ND the test and include the comment "Recent blood transfusion on record (≤30 days). Donor RBCs can mask a G6PD deficiency. Recommend testing 60 days after transfusion." Follow laboratory policy for notifying provider of a cancelled test.

2. If the patient has been transfused RBCs >30 <60 days prior, test the sample and include the comment "Interpret with caution, reported blood transfusion < 60 days."

C. Leukocytes and platelets may be rich in G-6-PDH and may cause interference in the assay if present beyond normal levels. In cases where the clinical picture fits G-6-PDH deficiency and the assay is not conclusive, a repeat with the buffy coat removed may be required.

XI. CALCULATIONS

N/A

XII. INTERPRETATION

A. Visually inspect the dried spots under long-wave ultraviolet light. Observe the amount of fluorescence in comparison to the Normal/ Deficient controls.

1. For a **NORMAL** sample, there is a distinct increase in fluorescence from 0-10 minutes.

2. For a **DEFICIENT** sample, there is no fluorescence demonstrated from 0-10 minutes. These are sent to the reference lab for quantitative testing.

3. **INCONCLUSIVE** results, in which fluorescence is in question, must be called "deficient" and sent to the reference lab for confirmatory/ quantitative testing.

XIII. RESULT REPORTING

Results are reported as either “Normal” or “Deficient”. When “Deficient” is entered, a G-6-PDA assay is automatically reflexed. These samples are given to the “send out” tech in SMS to be sent to the reference lab for quantitative testing.

XIV. TRAINING

Role	Training Needed
Testing Personnel	Competency Assessment
Trainer	Competency Assessment

A. To verify that an employee can perform a test according to procedure:

1. Review Procedure and Procedural Checklist, a step-by-step description of the test general guidelines of operation.
2. Complete demonstration of test by performing QC and/or patient test under the supervision of a qualified trainer. Document training on appropriate training documentation form and retain in employee file.

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