Procedure: BinaxNOW® G-6-PD Deficiency Screening Test
SH.CP.AU.hem.0133.0003

Original Author:	Effective (adopted) Date:	Supercedes Procedure #
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Version #	Approval Signature	Approval Date
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Version #	Distributed To	Date	# of Copies	Date of Removal
2	**QC, Lab Bench**		**1**	
2	**Sharepoint**		**1**	

Revised By	Date Revised	Effective (adopted) Date	Version #	Reason for Revision
ZEB	2/9/2018		.0002	Include new Deficient QC material
GMJ	3/25/2020		.0003	Add QC preparation, storage, and stability

Title: BinaxNOW® G-6-PD Deficiency Screening Test

I. PURPOSE

G-6-PD deficiency in red cells has been demonstrated to be the basis for certain drug-induced hemolytic anemias. Tarlov et al. points out the importance of identifying individuals with this biochemical defect as an aid in the selection of therapeutic agents. Severe hemolytic anemia may result in these individuals when they are given many commonly used drugs. The majority of subjects who have demonstrated G-6-PD 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.

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 BinaxNOW® G6PD (Glucose-6-Phosphate Dehydrogenase) Test is an *in vitro* enzyme chromatographic test for the qualitative detection of G6PD enzyme activity in human venous whole blood, collected in EDTA. The BinaxNOW® G6PD Test is a visual screening test used for differentiating normal from deficient G6PD activity levels in whole blood and is intended to aid in the identification of people with G6PD deficiency.

Samples which generate deficient results will be assayed at the reference laboratory using a quantitative G6PD test method to verify the deficiency.

II. SCOPE

This procedure will be used by technologists of the UR Medicine Hematology-Chemistry lab to perform the BinaxNOW® G6PD deficiency screen.

III. RESPONSIBILITIES

Roles	Responsibilities
Quality	Ensure that procedure is followed when performing the BinaxNOW® G-6-PD screening test.
Medical Director	Approval of the BinaxNOW® G-6-PD screening test procedure.
Management	Ensure that procedure is followed when performing the BinaxNOW® G-6-PD screening test.
Technologists	Follow procedure.

IV. ACRONYMS

UR	University of Rochester
SMH	Strong Memorial Hospital
G6PD	Glucose-6-Phosphate Dehydrogenase

V. SPECIMENS

A. **Specimen:** Venous blood collected by standard venipuncture procedure, into 1 EDTA tube. Minimum vol is 500µl, microtainer is acceptable. If blood is refrigerated, allow it to come to testing 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-PD, stored in refrigerator at 2-8◦C. Once reconstituted with 0.5mL of CLSI CLRW Type (or equivalent) water, the reagent is stable for one week when stored at 2-8◦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-PD, stored in refrigerator at 2-8◦C. Once reconstituted with 0.5mL of CLSI CLRW Type (or equivalent) water, the reagent is stable for one week when stored at -2-8◦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.

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 Material Safety Data Sheets in MSDS Manual for safety information.

VIII. MATERIALS

A. Supplies:

1. BinaxNOW® G6PD test kit (Catalog # 780-000) stored at room temperature.
2. CLSI CLRW Type (or equivalent) water

Component	Content	Quantity
Test Device	A cardboard, book-shaped, hinged test device containing the test strip.	25

Reagent A	Tris buffer containing detergent and red dye.	1
Sample preparation vials	Vials used to mix lysing reagent (Reagent A) with whole blood samples prior to transfer to the test devices.	25

3. Calibrated pipettes that deliver 70, 10, and 50 µl.
4. Calibrated room Thermometer
5. Calibrated Timer.

IX. PROCEDURE

A. Precautions:

- Leave test device sealed in its foil pouch until just before use, as the reagents on the test strip are light sensitive. Once removed from pouch, do not expose the device to direct sunlight or perform the test near a sunny window. Do not expose the device to fluorescent light for longer than 5 minutes, prior to testing.
- Do not mix components from different kit lots or shipments.
- The test must be performed at temperatures between 64°F to 77°F; failure to perform testing in the specified temperature range could lead to erroneous results. If the temperature is outside this range, DO NOT PERFORM THE TEST.
- Allow all samples, test devices, sample preparation tubes, and reagents to equilibrate to testing temperature (64°F to 77°F) before use.

1. Search each patient's history for RBC transfusions within the previous 60 days. See the Limitations section to confirm sample is acceptable for testing.

2. Check sample for clot. Partially clotted samples may be used as long as the hematocrit is ≥19 ≤53% after the clot has been removed. Fully clotted samples are not acceptable for testing.

3. Test the sample on the hematology analyzer to determine hematocrit and reticulocyte count. See the Limitations section to confirm sample is acceptable for testing. If Retic count is ≥5%, add comment @SG6R.

4. In your sample rack, place 1 preparation vial for each: Normal Control, Negative Control, and Patient(s).

5. Remove test device from foil pouch **just prior to use** and lay it flat on the work surface.
6. Label the front of each device.
7. Label each sample preparation vial and add 70µl of Reagent A.
8. Invert blood collection tube several times to mix sample before using.
9. Transfer 10µl of blood or control to the sample preparation vial containing the Reagent A.
10. Mix the blood or control sample in the Reagent A three times using a 50µl pipette by drawing and expelling the liquid from the tip. Use this lysed blood sample **immediately**.

11. See arrow on test device to find the White Sample Pad. **Slowly** add 50µl of the lysed blood sample to the middle of this pad. **Start 7 minute timer immediately after adding the sample to the pad.**
12. When the sample front **completely covers the top** of the reaction pad at the top of the test strip, peel off the adhesive liner from the right edge of the test device and close and securely seal the device.
13. Read test results **7 minutes** after sample is added to the Sample Pad. Results read before or after 7 minutes may be inaccurate.

X. LIMITATIONS

A. Abnormally low (17-18%) and high (54-65%) hematocrit levels can affect test performance. A low sample hematocrit increases the risk of a false deficient result for an otherwise normal sample because there are less red cells and hence less G6PD. Conversely, a similar situation can exist with a sample having a high hematocrit where a high number of red cells are present compared to a normal sample. In this case, a high hematocrit increases the risk of a false normal result for an otherwise deficient sample. If hematocrit is ≤ 18 or ≥ 54, .ND test and add comment “Low hematocrit interference” or “High hematocrit interference”. Follow laboratory policy for notifying provider of a cancelled test.

B. 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.

C. 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.”

XI. CALCULATIONS

None

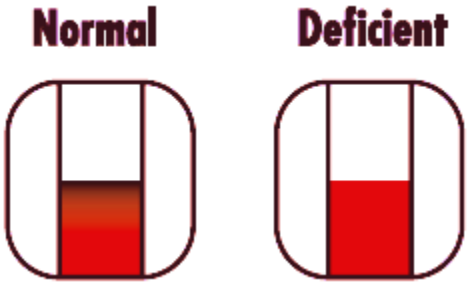
XII. INTERPRETATION

A. For a **NORMAL** sample, there is a distinct color change to black/brown in the top half of the reaction pad visible in the reading window. Note that the bottom of the pad visible in the reading window will be the color of the lysed blood sample.

B. For a **DEFICIENT** sample, there is **no** color change in the top half of the reaction pad at 7 minutes. These are sent to the reference lab for quantitative testing.

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

D. A test is **INVALID** if the sample front fails to completely cover the top of the reaction pad. Do not use the test. Repeat invalid tests with a new test device. Call Technical Service if the problem persists.



XIII. RESULT REPORTING

Results are reported as either "Normal" or "Deficient". When "Deficient" is entered, a G-6-PD 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.

XV. REFERENCES:

A. Harmening D.M.: Clinical Hematology and Fundamentals of Hemostasis. Second Edition, F.A. Davis Company, 1992, pp. 135-137.
B. Beutler E: A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency and glutathione reductase deficiency. Blood 28:553, 1966.
C. Kachmar JF, Moss DW: Enzymes. In Fundamentals of Clinical Chemistry. N W Tietz, Editor, Saunders, Philadelphia, 1976, pp 666-672.
D. Echler G: Determination of glucose-6-phosphate dehydrogenase. Am Journal of Medical Technology 49:259, 1983
E. Glucose-6-Phosphate Dehydrogenase Deficiency. In Hematology, W J Williams, E Beutler, A J Erslev, R W Rundles, Editors, McGraw-Hill, 1972, p 397.
F. BinaxNOW G6PD Product insert: PN IN780002 Rev.1 2013/05.

KNOWLEDGE CHECK
Binax NOW G-6-PD Screen

1.	The test device can be left out in direct light before testing.	T	F
2.	The test must be performed between 64°-77°F.	T	F
3.	For a **NORMAL** sample result there is a distinct color change to black/brown in the top half of the reaction pad.	T	F
4.	The test cannot be used to determine the degree of G6PD deficiency.	T	F
5.	When using blood drawn into an EDTA collection tube, the tube must contain ≥ 500µl.	T	F
6.	Abnormally low and high hematocrit levels do not affect the test performance.	T	F
7.	For a **DEFICIENT** sample result the color in the top half of the reaction pad is purple.	T	F
8.	A test is **INVALID** if the sample front fails to completely cover the top of the reaction pad.	T	F
9.	A reticulocyte count of 4% or greater requires a disclaimer.	T	F
10.	The test should be read after 7 minutes of incubation.	T	F

Any incorrect answers I may have initially written have been discussed and corrected. I now understand the answers I may have gotten wrong.

PASSING GRADE IS 80% OR GREATER

_____
Employee name (print)

_____ _____
Employee signature **(Date)**

_____
Supervisor/Manager name (print)

_____ _____
Supervisor/Manager signature **(Date)**