

**LIMITATIONS**

The BinaxNOW® G6PD Test is designed to distinguish normal levels of G6PD enzyme activity from deficient enzyme activity and cannot be used to assess the degree of deficiency. Samples which generate a deficient result on this test should be assayed on a quantitative G6PD test.

Abnormally low and high hematocrit levels can affect test performance. G6PD is normally found in the red blood cells, and a low hematocrit is an indicator that the number of red cells is low in a specific volume of blood. Therefore, 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.

**PERFORMANCE CHARACTERISTICS**

**Clinical Sample Correlation Study - BinaxNOW® G6PD Test versus Comparative Method**

The performance of the BinaxNOW® test was compared to a commercially available quantitative G6PD test in a prospective study conducted in 2007-2008 in the U.S. Both heparinized and EDTA whole blood specimens from 246 subjects were collected and evaluated.

The percent agreement of the BinaxNOW® G6PD test with the comparative method for detection of G6PD enzyme activity deficiency on both heparinized and EDTA blood samples is summarized below, including 95% confidence intervals.

**% AGREEMENT WITH HEPARIN SAMPLES:**

	Comparative Method		
	Deficient	Normal	
BinaxNOW®	48	4	
Test →	1	190	
	Total:	49	194

Deficient result percent agreement = 48 / 49 = 98.0% (CI = 89.3 - 99.6%)  
 Normal result percent agreement = 190 / 194 = 97.9% (CI = 94.8 - 99.2%)  
 Overall percent agreement = 238 / 243\* = 97.9% (CI = 95.3 - 99.1%)  
 (\* 3 invalid tests)

**% AGREEMENT WITH EDTA SAMPLES:**

	Comparative Method		
	Deficient	Normal	
BinaxNOW®	49	5	
Test →	1	191	
	Total:	50	196

Deficient result percent agreement = 49 / 50 = 98.0% (CI = 89.5 - 99.6%)  
 Normal result percent agreement = 191 / 196 = 97.4% (CI = 94.2 - 98.9%)  
 Overall percent agreement = 240 / 246 = 97.6% (CI = 94.8 - 98.9%)

**Interfering Substances**

The BinaxNOW®G6PD test was evaluated for possible interference from high levels of endogenous blood components. Whole blood samples were tested that contained bilirubin (conjugated and unconjugated), triglycerides, total cholesterol, lactic acid, lactate dehydrogenase, or glucose at concentrations above physiological levels. None of the endogenous blood components affected test performance. The presence of an elevated level of copper sulfate, which is known to inhibit G6PD enzyme activity, was also evaluated and did not affect test performance.

Blood samples with abnormally low (17-18%) and high (54-65%) hematocrit levels were evaluated, and test performance was affected as described in the Limitations section.

**Reproducibility Study - Multiple Operators**

A blind study of the BinaxNOW® G6PD Test was conducted at 3 separate sites using panels of blind coded specimens, which included G6PD normal and deficient samples. Participants tested each sample multiple times on 3 different days. There was 98% (123/125) agreement with expected test results, with no significant differences within run (replicates tested by one operator), between run (3 different days), between sites (3 sites), or between operators (6 operators).

**Precision Study - Single Operator**

Blood samples from two individuals were drawn into both EDTA and heparin collection tubes, and all 4 samples were tested in duplicate on the BinaxNOW® test on ten successive days by a single operator. The samples collected from one individual were interpreted as normal 100% of the time. The samples collected from the other individual were interpreted as deficient 100% of the time.

**REFERENCES**

1. Erbağci A.B. and N. Yilmaz. 2002. Erythrocyte Glucose 6-Phosphate Dehydrogenase Deficiency Frequency in Gaziantep, Turkey. *Eastern Journal of Medicine* 7 (1): 15-18.
2. Ernest Beutler. 1994. G6PD Deficiency. *Blood* 84 (11): 3613-3636.
3. Stiene EA. 1972. Red cell enzyme deficiencies: A Review. *Am J Med Tech.* 38:454.
4. Clinical and Laboratory Standards Institute (CLSI) Document - "Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture"

**ORDERING INFORMATION**

Reorder number: 780-000 G6PD Test kit

**Contact Information:**

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**BinaxNOW®  
G6PD  
Test**

For *In Vitro* diagnostic use.



**INTENDED USE**

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 heparin or ethylenediaminetetraacetic acid (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 should be assayed using a quantitative G6PD test method to verify the deficiency.

**SUMMARY AND EXPLANATION OF THE TEST**

G6PD is an enzyme that is part of the hexose monophosphate shunt and is the first enzyme of the pentose pathway. The enzyme is involved in the catalytic conversion of glucose to 6-phospho-gluconate, which produces an energy equivalent (NADPH) in the process.

Although much of the research on G6PD has focused on its function in red blood cells and its importance in cellular metabolism, it is equally important in providing defense mechanisms for erythrocyte membranes against oxidative stress. G6PD deficiency is the largest and most widespread enzymopathy in the world, affecting some 400 million people. There are approximately 200 variants and deficiency of the enzyme is frequently seen in males. The highest known gene frequency is 0.65 among Kurdish Jews. Prevalence is approximately 21% in West Africa and 11% in some Asian countries such as Thailand.<sup>1</sup> In middle and northern Europe the frequency of G6PD deficiency is about 0.0005. In the United States, the gene frequency of enzyme deficiency is 10 - 11% among African American males.<sup>2</sup>

When strong oxidizing agents such as those found in many commonly used drugs (anti-malarial drugs, sulfa drugs, and ascorbic acid)<sup>3</sup> are administered, a deficiency in red cell G6PD does not allow for the production of sufficient reducing equivalents



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to prevent clinical complications such as acute spherocytic hemolytic anemia. It is therefore important that individuals with this deficiency be identified prior to the use of certain therapeutic agents.

The BinaxNOW® G6PD Test is a simple, rapid test for the detection of G6PD enzyme activity using whole blood collected by venous draw. The BinaxNOW® test takes less than 10 minutes per sample to complete, does not require the use of special equipment, and the reagents are provided ready to use and can be stored at room temperature.

## PRINCIPLES OF THE PROCEDURE

The BinaxNOW® G6PD test device consists of a lateral flow test strip comprised of a white sample pad and a reaction pad, which is located at the top of the strip (2 U.S. patents pending). The reaction pad contains the reagents necessary for the G6PD enzymatic reaction and the subsequent reduction of a nitro blue tetrazolium dye into its concomitant blue formazan product. The resulting color change on the strip indicates enough G6PD activity is present to presume the sample is not deficient.

To perform the test, a whole blood sample is mixed with red blood cell (RBC) lysing reagent in a sample preparation vial and then transferred to the test device sample pad. The lysed blood sample migrates up the test strip, reconstituting reagents in the reaction pad. When the sample front (or liquid migration) covers the entire reaction pad, the device is closed.

Test results are read visually. If no change in the red color of the sample front is observed at the test read time, the sample is presumed to be deficient in G6PD enzyme activity. Samples normal in G6PD activity produce a distinct color change - the red sample color changes to a brown / black color on the upper half of the reaction pad.

## REAGENTS AND MATERIALS

Materials Provided

### BinaxNOW® G6PD Test Kit:

- Test Devices: A cardboard, book-shaped, hinged test device containing the test strip
- Reagent A: Tris buffer containing detergent and red dye
- Sample preparation vials: Vials used to mix lysing reagent (Reagent A) with whole blood samples prior to transfer to the test devices

## MATERIALS REQUIRED BUT NOT PROVIDED

- G6PD Normal Quality Control (pool of 3 - 5 heparin or EDTA whole blood samples)
- G6PD Deficient Quality Control (heparin or EDTA whole blood sample that is deficient in G6PD enzyme activity - see the Quality Control section for preparation instructions)
- Standard blood drawing equipment, clock, timer or stopwatch
- Calibrated pipettes capable of delivering 10 µl, 50 µl and 70 µl volumes
- Calibrated thermometer

## PRECAUTIONS

1. For *in vitro* diagnostic use.
2. **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.**
3. Do not use kit past its expiration date.
4. Do not mix components from different kit lots.
5. **The test must be performed at temperatures between 18-25°C (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.**
6. Allow all samples and reagents to equilibrate to testing temperature before use.
7. Mix whole blood sample well by inverting the tube or vial several times, and before sampling, prime the pipette tip by drawing the sample into the tip and expelling it.
8. Patient samples and test devices should be handled as though they are capable of transmitting disease. Observe established precautions against bloodborne pathogens. Do not reopen or reuse test cards.
9. When interpreting test results, use a bright light.
10. **The test read times are different for samples collected in heparin and EDTA blood tubes. For all heparin samples, read test results 5 minutes after sample is added to the Sample Pad. For all EDTA samples, read test results 7 minutes after sample is added to the Sample Pad.**
11. When using blood drawn into EDTA tubes, ensure that the collection tube is completely filled as under filled tubes could have an incorrect blood-to-additive ratio, and the chelating effect of EDTA on magnesium chloride may generate a false deficient test result.
12. All pipette tips and sample preparation vials are single use items.
13. Contamination of dispensing equipment, containers or reagents can lead to inaccurate results.

## STORAGE AND STABILITY

Store kit at room temperature (15-30°C, 59-86°F). The BinaxNOW® G6PD Test Kit and reagents are stable until the expiration dates marked on their outer packaging and containers when stored as specified.

## QUALITY CONTROL

### External G6PD Deficient and Normal Controls:

Good laboratory practice recommends that deficient and normal controls be run with each new shipment or lot to ensure that:

- test reagents are working, and
- the test is being correctly performed

For a G6PD normal control, a pool of equal volumes of 3 - 5 heparin or EDTA whole blood samples from G6PD presumed normal individuals can be used. A normal control pool is stable for 7 days at 2-8°C.

To prepare a G6PD deficient control, follow the instructions below:

1. Place a minimum of 3 mls of heparin or EDTA whole blood into a centrifuge tube and spin at 1500 x g for 5 minutes. (NOTE: The blood should be no more than 3 days old.)
2. Carefully remove all plasma. Measure amount removed.
3. Replace plasma with an equal volume of high purity or deionized water.
4. Gently mix sample by inverting / rotating for 15 minutes.
5. Place sample in 50°C water bath for 4 hours. Ensure that the water level in the bath is higher than the level of blood in the tube.
6. Mix well for at least 10 seconds with vortex.
7. Test the processed blood on the BinaxNOW® G6PD test to verify that it produces a deficient result.
8. Aliquot this G6PD deficient control into appropriately sized and labeled containers or tubes.
9. Deficient control, prepared as described above, has been shown to be stable for 7 days at 2-8°C and for up to 6 months when stored at ≤-20°C (non-frost free freezer). Each lab should establish its own stability criteria for its G6PD deficient control because of the possible biological variability of whole blood.

Other controls must be tested in order to conform with:

- local, state and/or federal regulations,
- accrediting groups, and/or,
- your laboratory's standard Quality Control procedures

Refer to 42 CFR 493.1256 for guidance on proper QC practices (U.S. customers only).

If the correct control results are not obtained, do not report patient results. Contact Technical Service during normal business hours (EST).

## SPECIMEN COLLECTION AND HANDLING

Collect venous blood, by standard venipuncture procedure<sup>4</sup>, into a heparin or EDTA tube. Test whole blood samples as soon as possible after collection. If the test cannot be performed immediately, blood samples may be held up to one week refrigerated (2-8°C). **Do not freeze samples prior to testing.**

If blood is refrigerated, allow it to come to testing temperature and mix well prior to testing. If G6PD quantitative test confirmation of a BinaxNOW® deficient test result is necessary on a sample that has been stored, appropriate criteria for the sample handling and storage requirements used for that testing should be followed.

## TEST PROCEDURE

See the Specimen Collection and Handling section for information regarding sample collection.

**Important:** Allow all samples and reagents to equilibrate to testing temperature (18°- 25°C) before use.

**Devices should be removed from protective pouches and tested immediately.** Once removed from the pouches, do not expose the devices to sunlight. Do not expose the devices to fluorescent light for longer than 5 minutes, prior to testing.

1. Remove device from foil pouch **just prior to use** and lay it flat on the work surface.
2. Record the room temperature on the front of the device. If the temperature is outside 18°C to 25°C, **DO NOT PERFORM THE TEST.**
3. Add 70 µl of Reagent A to a sample preparation vial.
4. Invert blood collection tube several times to mix sample before using.
5. Transfer 10 µl of blood to the sample preparation vial containing the Reagent A.
6. Mix the blood sample in the Reagent A three (3) times using a 50 µl pipette by drawing and expelling the liquid from the tip. Use this lysed blood sample **immediately.**
7. See arrow on test device to find the White Sample Pad. **Slowly** add 50 µl of the lysed blood sample to the middle of this pad. **Start timer immediately after adding the sample to the pad.**
8. 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.
9. **For all heparin samples**, read test results **5 minutes** after sample is added to the Sample Pad. Results read before or after 5 minutes may be inaccurate. **For all EDTA samples**, read test results **7 minutes** after sample is added to the Sample Pad. Results read before or after 7 minutes may be inaccurate.

Note: When reading test results, use a bright light.

## RESULT INTERPRETATION

### NORMAL



### DEFICIENT



### For Heparin Samples →

For a **NORMAL** sample, **within 5 minutes** 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.

For a **DEFICIENT** sample, there is **no** color change in the top half of the reaction pad **at 5 minutes**. Samples in which a color change is in question **must be called DEFICIENT**.

### For EDTA Samples →

For a **NORMAL** sample, **within 7 minutes** 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.

For a **DEFICIENT** sample, there is **no** color change in the top half of the reaction pad **at 7 minutes**. Samples in which a color change is in question **must be called DEFICIENT**.

### For both sample types →

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.