

Glucose-6-Phosphate Dehydrogenase (G-6-PDH) Deficiency

REF 203-A

For other languages
Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue

Para outras línguas
For andre språk


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INTENDED USE

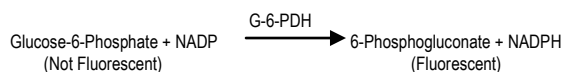
Trinity Biotech Glucose-6-Phosphate Dehydrogenase reagents are for the qualitative, visual fluorescence screening of G-6-PDH in whole blood. Samples which have been determined deficient or intermediate should be assayed by a quantitative G-6-PDH method such as **Trinity Biotech** Procedure No. 345.

BACKGROUND AND PRINCIPLE OF TEST

G-6-PDH deficiency in red cells has been demonstrated to be the basis for certain drug-induced haemolytic anaemias.¹ Tarlov et al.¹ points out the importance of identifying individuals with this biochemical defect as an aid in the selection of therapeutic agents. Severe haemolytic anaemia may result in these individuals when they are given many commonly used drugs. 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).² This defect should be considered whenever an otherwise unexplained case of haemolytic anaemia 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.^{1,3,4}

For semi-quantitative purposes, Beutler³ first suggested estimating glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) in terms of visual appearance of fluorescence in red cell-substrate mixtures. The **Trinity Biotech** procedure is a modification of the Beutler revised method,^{5,6} involving the reaction:



The test is performed by incubating a small amount of blood with glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate (NADP). Drops of the mixture are removed at 5-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.

REAGENTS

TRIZMA® BUFFER SOLUTION, 1 x 12 ml, 203-2A
TRIZMA® Buffer, 100 mmol/L, pH 7.8 and preservative.

G-6-PDH SUBSTRATE, 5 x 2 ml, 203-2B
Glucose-6-Phosphate (4 µmol), NADP (1.6 µmol), Glutathione, oxidized (1.6 µmol), and lytic agent.

PRECAUTIONS

Glucose-6-Phosphate Dehydrogenase Deficiency reagents are "For *in vitro* diagnostic use". Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.
For professional use only.
In case of damage, do not use.

PREPARATION

G-6-PDH Substrate solution is prepared by reconstituting G-6-PDH Substrate vial, Catalogue No. 203-2B, with 2.0 ml TRIZMA® Buffer Solution, Catalogue No. 203 2A. Allow to stand for 1-2 minutes and then mix by inversion.

STORAGE AND STABILITY

Store G-6-PDH Substrate refrigerated (2-8°C). Reagent label bears expiration date.

Store TRIZMA® Buffer Solution refrigerated (2-8°C). Discard if turbidity develops.

G-6-PDH Substrate solution is stable for 2 weeks stored frozen (≤ -20°C), 1 week stored refrigerated (2-8°C), or up to 4 hours at room temperature (18-26°C).

DETERIORATION

If a dried spot of G-6-PDH Substrate solution exhibits fluorescence when viewed under long-wave ultraviolet light, or blood-reagent spots prepared from normal specimens yield dull fluorescence, the reagent may have deteriorated and should be discarded.

OPTIONAL REAGENTS

G-6-PDH CONTROLS

G-6-PDH Control Normal, 6 x 0.5 ml, G6888
G-6-PDH Control Intermediate, 6 x 0.5 ml, G5029
G-6-PDH Control Deficient, 6 x 0.5 ml, G5888

Lyophilized preparations containing G-6-PDH in a stabilized human red cell haemolysate base.

G-6-PDH Controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled observing the same safety precautions employed when handling any potentially infectious material.

Quality controls should return their expected result, otherwise the test run is invalid.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A4.⁷ No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Whole blood collected with ethylenediaminetetraacetic acid (EDTA), heparin or acidcitrate-dextrose (ACD) is satisfactory. Red cell G-6-PDH is stable in whole blood for one week refrigerated (2-8°C), but is unstable in red cell haemolysates.² Freezing of blood is not recommended.

INTERFERING SUBSTANCES

The results of this assay should be interpreted with the haematologic status of the individual in mind at the time of testing.

Leukocytes and platelets may be rich in G-6-PDH and may cause some interference in the assay if present beyond normal levels.⁸ Therefore, higher than normal levels of G-6-PDH could occur in cases of leukocytosis and thrombocytosis. In cases where the clinical picture fits G-6-PDH deficiency and the assay is not conclusive, the treating clinician may, on an individual basis, want to request a repeat of the assay with the buffy-coat removed before preparing the red cell haemolysate.

Young red blood cells (reticulocytes) have higher G-6-PDH levels than mature erythrocytes. A false-negative result, indicating the sample is normal, may be obtained in individuals with an anaemia and resulting reticulocytosis. Therefore the screening test should be performed after resolution of reticulocytosis resulting from a haemolytic episode or other causes.⁹ The clinician should decide the time interval to wait for testing since it will depend on individual circumstances.

We are not aware of any drugs which may interfere with the test.

PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

A long-wave ultraviolet light in a viewing box or a darkened room is needed.
A suitable lamp is the General Electric No. F15T8-BL, 15W, black light, which emits light between 320-420 nm
A short-wave ultraviolet light should not be used
Conventional or automatic pipettes are needed that reliably deliver 0.01, 0.2 and 2.0 ml
Pasteur pipettes or small glass rods are used to transfer reaction mixture
Whatman No. 1 filter paper
37°C Water bath
Timer
Recently drawn normal whole blood sample stored in refrigerator (less than one week)

PROCEDURE

- Into tube (e.g., 13 x 100 mm) labeled NORMAL, add 0.2 ml G-6-PDH Substrate solution and 0.01 ml recently drawn normal blood. Mix by swirling and promptly transfer a drop of mixture to filter paper (Whatman No. 1). Identify spot on filter paper as "Zero-Time Normal". Place NORMAL tube in 37°C water bath and record time.
Note: Spot sizes should be approximately 1/2 inch in diameter.
- Into tube labeled TEST, add 0.2 ml G-6-PDH Substrate solution and 0.01 ml blood sample to be tested. Mix by swirling and promptly transfer a drop of mixture to filter paper. Identify spot on filter paper as "Zero-Time Test". Place TEST tube in 37°C water bath and record time.
- Transfer additional drops of NORMAL and TEST to filter paper 5 and 10 minutes after "Zero-Time" applications. Label spots with appropriate times and allow to dry for 15-20 minutes.
- Visually inspect dried spots under long-wave ultraviolet light. Record fluorescent intensity (absent, weak, moderate or strong) of each sample at 5 and 10 minutes.
Notes:
 - Because of the rapid speed of reaction, "Zero-Time" spots may exhibit traces of fluorescence.
 - Fluorescent spots are stable for up to two weeks stored in a plastic bag with desiccant in the refrigerator at 2-8°C.
 - In the absence of a recently drawn blood sample required for the Normal tube in Step 1 of the Procedure, you may substitute G-6-PDH Normal Control, Catalogue No. G6888.

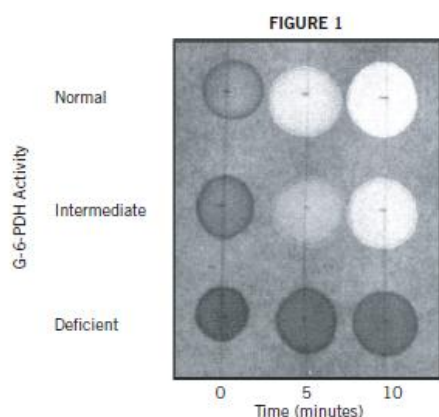
QUALITY CONTROL

Samples with normal G-6-PDH and with G-6-PDH deficiency should be included with each group of assays to ensure reliable test performance. A sample with intermediate G-6-PDH activity could also be included. G-6-PDH Control Normal, Catalogue No. G6888, G-6-PDH Control Intermediate, Catalogue No. G 5029, and G-6-PDH Control Deficient, Catalogue No. G 5888, are suitable for this purpose. They are lyophilized human blood preparations with known levels of G-6-PDH activity.

RESULTS

The test is designed to distinguish normal from grossly deficient samples. By visually comparing the amount of fluorescence in the 5 minute spots of the sample with that of a normal sample, samples with intermediate deficiencies may also be discerned.

Typical results are shown in Figure 1 below:



A normal sample will demonstrate moderate to strong fluorescence after 5 minutes, and strong fluorescence after 10 minutes.

An intermediate level sample, will generally demonstrate weak/very faint fluorescence after 5 minutes and moderate fluorescence after 10 minutes.

A grossly deficient sample will reveal weak/very faint or no fluorescence even after 10 minutes.

It is recommended that samples which have been determined as deficient or intermediate by this procedure be assayed by a quantitative G-6-PDH technique such as **Trinity Biotech** No. 345.

LIMITATIONS

The test is designed to distinguish normal and intermediate from grossly deficient samples and should not be used to assess the degree of deficiency. It is recommended that samples which have been determined as deficient or intermediate by this Procedure be assayed by a quantitative G-6-PDH technique such as **Trinity Biotech** Procedure No. 345.

EXPECTED VALUES

Blood samples from 100 clinically healthy adults showed moderate to strong fluorescence after 5 minutes and strong fluorescence after 10 minutes. Blood samples from 10 donors with intermediate G-6-PDH showed moderate fluorescence after 5 minutes and moderate fluorescence after 10 minutes. Blood samples from 25 known G-6-PDH deficient individuals showed no fluorescence after 5 minutes and 10 minutes.

PERFORMANCE CHARACTERISTICS

CORRELATION

125 samples including normal and deficient enzyme levels were assayed by the G-6-PDH Deficiency Kit 203A, the Trinity Biotech G-6-PDH Kit 400K and an approved quantitative test kit. All samples were identified similarly by the three test kits. A further 10 intermediate enzyme levels samples were assayed simultaneously by G-6-PDH Deficiency Kit 203A and an approved quantitative test kit. All samples were identified similarly by the two test kits.

REPRODUCIBILITY STUDIES

Normal, deficient, and intermediate samples were assayed on three occasions over a period of several days. Results obtained for each of the samples were identical for the replicate assays.

REFERENCES

1. Tarlov AR, Brewer GJ, Carson PE: *Primaquine sensitivity*. Arch Int Med 109:209,1962.
2. Stiene EA: *Red cell enzyme deficiencies: A Review*. Am J Med Tech 38:454, 1972.
3. 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.
4. Beutler E: *Red Cell Metabolism*. Grune & Stratton, New York, 1971, p 117.
5. Beutler E: *Special modifications of the fluorescent screening method for glucose-6-phosphate dehydrogenase deficiency*. Blood 32:816 1968.
6. Beutler E, Blume KG, Kaplan C, Lohr W, Ramot B, Valetine WN: International committee for standardization in haematology: *Recommended screening test for glucose-6-phosphate dehydrogenase (G-6-PD)*. Bri J of Haem, 43:469 - 477, 1979.
7. Clinical and Laboratory Standards Institute (CLSI). *Protection of laboratory workers from occupationally acquired infections; Approved guideline—Fourth Edition*. CLSI document M29-A4 Vol. 34, No. 8, 2014.
8. Echler G: *Determination of glucose-6-phosphate dehydrogenase*. Am J Med Technol 49:259, 1983.
9. *Glucose-6-Phosphate Dehydrogenase Deficiency*. IN Hematology, WJ Williams, E Beutler, AJ Erslev, RW Rundles, Editors, McGraw Hill, 1972, p 397.

ORDERING INFORMATION











KIT	
Catalogue No.	203-A
Maximum Assays	50
Contents – Catalogue Numbers	
TRIZMA® Buffer, 203-2A	12 ml
G-6-PDH Substrate, 203-2B	5 x 2 ml

Reagents available in kit only and cannot be purchased individually.

OPTIONAL REAGENTS

Catalogue No.	Item	Quantity
G6888	G-6-PDH CONTROL NORMAL	6 x 0.5 ml
G5029	G-6-PDH CONTROL INTERMEDIATE	6 x 0.5 ml
G5888	G-6-PDH CONTROL DEFICIENT	6 x 0.5 ml

GUIDE TO SYMBOLS

 Consult instructions for use	 Temperature limit
 Catalogue number	 <i>In vitro</i> diagnostic medical device
 G-6-PDH Substrate	 Batch Code
 TRIZMA® Buffer Solution	 Manufacturer
 www.trinitybiotech.com eIFU indicator	 Use-by date



Trinity Biotech plc,
IDA Business Park
Southern Cross Road
Bray, Co. Wicklow
Ireland
Tel. 353 1 2769800
Fax. 353 1 2769888
www.trinitybiotech.com

USA ENQUIRIES:
Trinity Biotech USA
2823 Girls Road,
Jamestown, NY 14701.
Tel. 1800 325 3424
Fax. 716-488-1990

