Atlas Medical

G6PD Kit

A Screening Kit for Dried Blood Spots & Whole Blood Samples

This kit is particularly suitable for screening for G-6-PD deficiency in newborns.

Procedure

The assay procedure is according to reaction described by Beutler (1,2).
The enzyme determined is glucose-6-phosphate dehydrogenase which is abbreviated either as G-6-PD or G-6-PDH.

Test principle

The NADPH produced in the reaction fluoresces under long-wave UV-light. If there is a marked deficiency of this enzyme, or if G-6-PDH is lacking entirely, no fluorescence will be observed.

Sample material

- Whole blood dried on filter paper Apply a drop of blood to absorbent paper provided with kit and let dry completely (stable for one week at 20-25°C).
- Whole blood.

Whole blood may be used instead of dried blood. Heparin, citrate, oxalate and EDTA are suitable anticoagulants. The blood specimens are stable for seven days at most. Use 0,005 ml (5 microliters) for the assay.

Reagents

Contents of solution	Concentration in the test
Glucose-6-phosphate	1 mmol/l
NADP	0,75 mmol/l
GSSG (oxidized glutathione)	0,8 mmol/l
Saponin	0,2%
Tris(hydroxymethyl)-aminomethane	225 mmol/l, pH 7.8

Preparation and stability of reagent solution

1. Dissolve the contents of one vial containing the freeze dried powder (Reagent vial) with the appropriate amount of Dilution buffer (mentioned on the reagent vial). Stable for four weeks at +4°C or two months at -20°C

Sample preparation

Punch out a disk of blood-stained paper of 5 mm diameter (3 mm can also be used).

Procedure

Introduce into a vial (volume 1-3 ml)	
One blood disk	5 mm diameter
Reagent solution	0,1 ml
Mix well, incubate for 10 minutes at 25°C and then apply 0,01 ml of the test solution to a new filter paper	

Evaluation

When the filter paper is dry (approximately after 1 hour), view under a long-wave UV-lamp in a darkened room. Samples obtained from normal or slightly reduced G-6-PDH activity will show strong fluorescence. Failure to fluoresce after 10 minutes incubation suggests a total or marked deficiency of G-6-PDH.

Notes

1. In some forms of G-6-PDH deficiency, young erythrocytes manifest normal enzyme activity. Blood from patients who have just experienced a hemolytic crisis must first be treated by the procedure described by Herz et al (4) to separate the older erythrocytes from the prevailing population of young ones. Use 0,005 ml of the suspension so obtained for the assay.
2. If the patient has received a blood transfusion, this test is clinically significant only after 30 days have elapsed, because the donor's erythrocytes generally manifest a normal G-6-PDH activity and can thus bias the result before the expiration of this time.
3. Any commercially available UV-lamp emitting long-wave UV-light is adequate for the evaluation.

Warning: The Dilution buffer contains sodium azide as preservative. Do not swallow. Avoid contact with the skin and mucous membranes.

References

1. Beutler E. Drug-induced hemolytic anemia and non-spherocytic hemolytic anemia. In Glucose-6-Phosphate Dehydrogenase (Yoshida A. and Beutler E., Eds) pp. 3-12, Academic, Orlando.
2. Beutler E. A series of new screening procedures for pyruvate kinase deficiency, glucose – 6 – phosphate dehydrogenase deficiency and glutathione reductase deficiency. *Blood* 1966;28:553-562.
3. Dow PA, Petteway MB, Alperin JB. Simplified method for G6PD screening using blood collected on filter paper. *Am J Pathol* 1974;61:333-336.
4. Herz F, Kaplan E, Scheye ES. Diagnosis of erythrocyte glucose-6-phosphate dehydrogenase deficiency in the negro male despite hemolytic crisis. *Blood* 1970;35:953-954.

GENERAL REFERENCE

Luzzatto L, Mahta A. Glucose 6-Phosphate Dehydrogenase Deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D eds. The Metabolic and Molecular Basis of Inherited Disease. USA: McGraw-Hill Publishers, 1995:3367-3398.

Atlas Medical
William James House
Cowley Rd, Cambridge, CB4 0WX
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524

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