Screening for G. 6-P.D. Deficiency

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IN MALAYSIA AND SINGAPORE, red cell glucose-6-phosphate dehydrogenase (G.6-P.D.) deficiency is common (Vella, 1959; Wong, 1974, 1975). It is a major cause of neonatal hyperbilirubinaemia and kernicterus in this region (Wong, 1964, 1965; Sinniah, 1971), in addition to being responsible for drug induced haemolytic anaemia. It is most important to detect G.6-P.D. deficiency in newborns in the population so that morbidity and mortality from neonatal hyperbilirubinaemia may be reduced. Many tests for G.6-P.D. deficiency have been devised. Of these, the earlier Heinz body test (Beutler, 1955) and the glutathione stability test (Beutler, 1957) were too non-specific for screening purposes. The more specific and highly sensitive tests, which have been used require specialized equipment, are very tedious for screening and are reserved for detecting mildly deficient heterozygotes. These include spectrophotometric assay of the enzyme; methaemoglobin elution test and the G.6-P.D. tetrazolium cytochemical method. Screening tests were developed which are equally specific but are much less sensitive, viz, brilliant cresyl-blue method (Motulsky, 1961); methaemoglobin reduction test (Brewer, 1962) and the MTT spot test (Fairbanks, 1962). These however are not entirely satisfactory for screening G.6-P.D. deficiency in Malaysia, especially in the rural areas where difficulties may arise in the collection and despatch of blood specimens to the nearby hospital.

In 1966, Beutler devised a fluorescent screening spot test based on the fact that reduced nicotinamide adenine dinucleotide phosphate (NADPH) fluoresces under ultra violet light. In the presence of G.6-P.D. glucose-6-phosphate (G-6-P) is oxidised to 6-phosphogluconate, this being associated with the reduction of NADP to NADPH which fluoresces (Figure 1). With G.6-P.D. deficiency NADPH is not produced in sufficient quantities to cause fluorescence. Beutler (1968) improved its sensitivity by adding oxidised glutathione (GSSG) to the

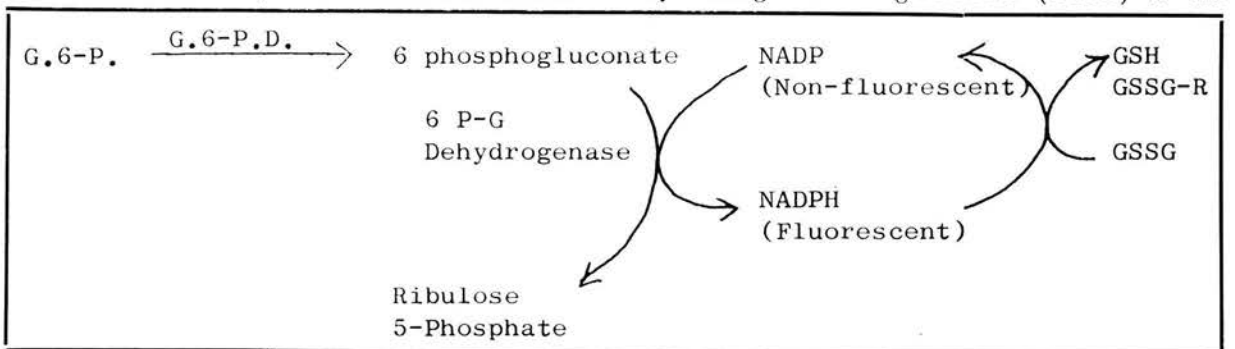

Fig. 1 Part of Hexose-Monophosphate Shunt to illustrate the principle underlying the fluorescent screening test for G. 6 - P. D. deficiency.

reaction mixture, eliminating almost entirely the slight fluorescence which sometimes appears in the presence of mild G.6-P.D. deficiency. When GSSG is present, NADPH is oxidised through the action of glutathione reductase (GSSG-R). He also enhanced the initial intensity and stability of the fluorescence by substituting tris-hydro-chloride buffer for phosphate buffer.

White (1972) reported that the test could be accurately performed with blood applied on blotting paper instead of being collected in a tube or bottle. This clearly simplifies the collection and despatch (to a distant laboratory, if necessary) of such blood specimens.

We have found this modified fluorescent spot test to be very useful for screening G.6-P.D. deficiency in Malaysia.

Method

A. Specimen collection

Whole blood is applied directly to the end of a filter paper strip, allowing it to soak through completely. Sufficient blood is smeared to allow a $\frac{1}{4}$ inch diameter disc of the blood-soaked paper to be punched out. Preferably one inch of the filter paper should be blood-filled to enable subsequent checking, if necessary. The filter strip is then mounted on a data card as shown (Fig. 2).

When the specimen is air dried, it may be stored for at least two weeks so that results may be checked if necessary or performed when convenient if results are not urgently required. Storage for this period of time has not reduced the accuracy of the test.

B. Reaction mixture:

This is made up of equal parts of the following:

1. Glucose-6-phosphate (G-6-P) 0.01 molar solution (molecular weight 358.2) in tris-hydrochloride buffer, 0.75 molar solution (molecular weight 121.1).
2. GSSG (oxidised glutathione) 0.008 molar solution (molecular weight 612.7).
3. NADP – 0.0075 molar solution (molecular weight 765.44). This should preferably be freshly prepared though when stored deep frozen, it remains stable for not less than one week.

0.1 ml of the reaction mixture is placed in a 10 × 75 mm labelled test tube. A $\frac{1}{4}$ inch single-hole paper punch is used to punch out a disc of blood-soaked paper which is then placed in the reaction mixture and incubated at 37°C for 15 minutes.

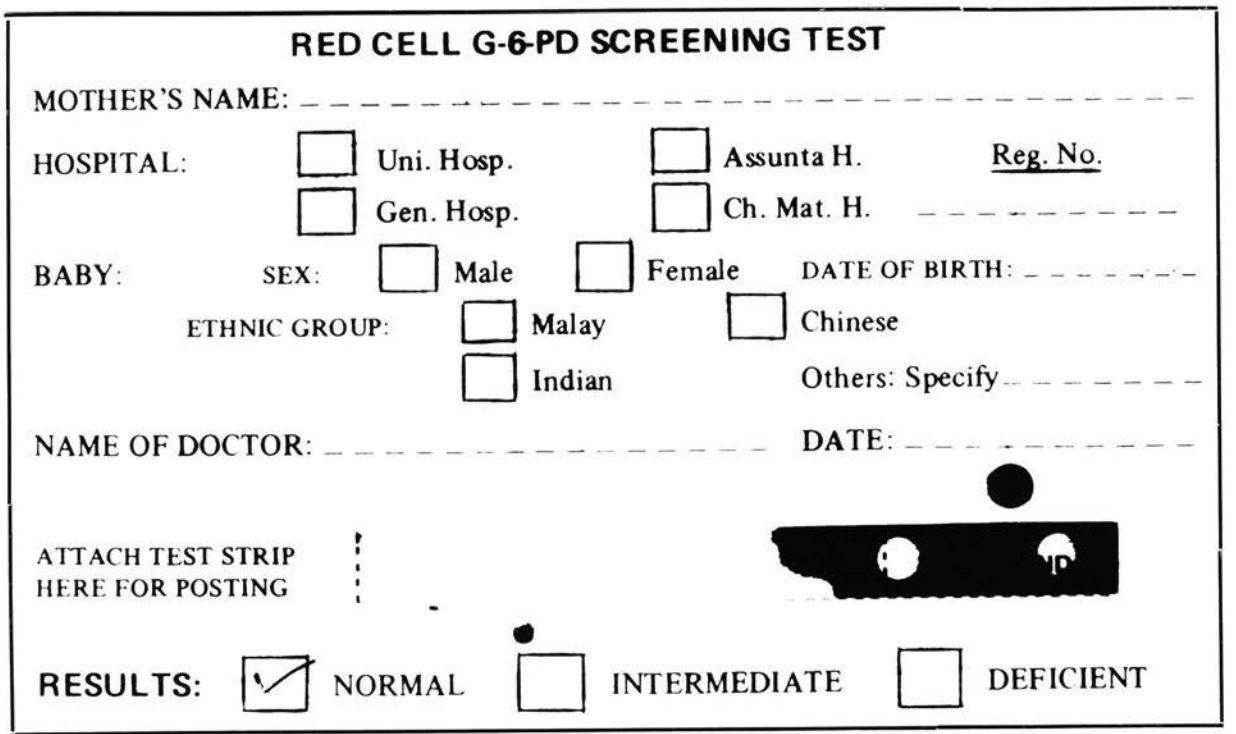

Fig. 2 Data card sample showing attached filter paper strip with punched-out discs.

Using a capillary tube, the test mixture is spotted on a filter paper and allowed to dry after which it is examined under a source of long ultra-violet light for fluorescence. It is most important to allow the spot to dry thoroughly to obtain suitable fluorescence. Large numbers of similar test mixtures may be spotted at the same sitting.

Interpretation

Spots which contain normal G.6-P.D. activity fluoresce brightly under ultra-violet light, contrasting sharply with those deficient in G.6-P.D. which show almost no fluorescence. Those intermediate in G.6-P.D. activity fluoresce less brightly. It is to be noted that this test has not been evaluated for heterozygote detection (Beutler, 1966).

Using the above test, the incidence of G.6-P.D. deficiency was determined on 2,049 cord blood specimens, collected as described and despatched by post from four other hospitals in Kuala Lumpur and Petaling Jaya. The results are shown in Table I.

Comments

The test described requires only one or two drops of blood (cord blood or blood from a heel prick) and the collection of specimens is simple and convenient. However, by far the greatest advantage of the test is that specimens can be despatched from outlying areas by post as they remain stable for as long as 3 weeks. In Malaysia, many deliveries are still conducted at home, often in remote rural areas where nearby hospital facilities are not available. It is not uncommon to find that babies born in these areas develop hyperbilirubinaemia due to G.6-P.D. deficiency and that kernicterus is not a rare complication. As it is possible to reduce morbidity and mortality from this cause by avoidance of certain "trigger" drugs, phototherapy, exchange transfusion and genetic counselling, this high-risk group must first be identified. The method described, because of its simplicity, is particularly suitable for use in remote rural areas to detect G.6-P.D. deficiency. Cord blood may be collected as described with the help of midwives (bidans) who may then despatch them to an appropriate centre. Infants who are found to be G.6-P.D. deficient can then be rapidly identified and appropriately treated.

The test itself is simple to perform. The only "specialized" equipment required is the long-wave ultra-violet lamp which is inexpensive if bulk purchases are made. It is estimated that each test costs between Malaysian five and ten cents. Up to two hundred specimens can be tested in less than half an hour, inclusive of the time required to prepare the reagents. Its reliability has been tested elsewhere (Beutler, 1966; White, 1972; Fairbanks, 1969). Fairbanks et al. (1969) in an analysis of published methods of detecting G.6-P.D. deficiency found that the fluorescent spot test is as specific and sensitive as the other currently available screening test, for example the Motulsky's dye – decolorisation method. The incidence of G.6-P.D. deficiency in cord blood of newborns among the major races in Kuala Lumpur and Petaling Jaya as detected by the fluorescent screening test is similar to that reported by Wong (1964) in Singapore using the brilliant cresyl blue decolorisation method (Table II). It is higher among the Chinese (3.1%) and the Malays (1.4%) than among the Indians (0.2%). We have also compared this test with the Prankerd's modification of Motulsky's dye decolorisation test (1962) on 20 known G.6-P.D. deficient patients and 20 other G.6-P.D. normal subjects and have not found any discrepancy in the results.

Conclusion

This is a simple, cheap, reliable and rapid test for G.6-P.D. deficiency. We recommend it as a screening test, especially for newborns and is particularly suitable for us in remote rural areas in Malaysia.

Table I

Incidence of G.6-P.D. deficiency in cord blood of newborns in Kuala Lumpur and Petaling Jaya as detected by the Fluorescent Screening Test

	Total tested	G.6-P.D. Deficient				Female heterozygotes
		Males	Females	Total	%	
Chinese	931	26	3	29	3.1	5
Malays	629	9	0	9	1.4	4
Indians	487	1	0	1	0.2	—
Others	2	2	0	2	—	—

Table II
Incidence of G.6-P.D. deficiency in Malaysia and Singapore (%).

	Wong (1964)	Robinson, Lau, Lin & Chan (1975)
Chinese	1.9	3.1
Malays	1.5	1.4
Indians	0.5	0.2
Aborigines	–	–

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