

A FREEZE-DRIED METHOD FOR PREPARATION OF G6PD REAGENT TUBES

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Summary

Mixed reagents for the Glucose-6-phosphate dehydrogenase (G6PD) deficiency fluorescent screening test were freeze-dried in plastic tubes. The reagents were then reconstituted with distilled water and the test was performed in the usual way. Initial testing with the freeze-dried mixed reagents gave consistent positive reaction to 12 normal blood samples and negative reaction to 9 G6PD deficient blood samples. This will enable a laboratory with freeze-drying facilities to prepare reagent tubes in bulk. As these tubes can be kept at 4°C and do not require to be stored at -20°C, a major laboratory can prepare these tubes and supply small laboratories for screening purposes.

Key words: G6PD deficiency, freeze-dried reagents, screening test

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited disorder in Malaysia. It was estimated to occur in up to 25% of the male population in South-East Asia.¹ Our laboratory, which serves the Kuala Lumpur General Hospital which has a delivery rate of approximately 24,000 a year, records an incidence rate of 7.5% in the male babies.

The mixed reagents for the fluorescence-based screening test for G6PD can be stored at -20°C for at least 3 years and at 4°C for at least 2 months.² In our laboratory, separate stock solutions estimated to last one week are prepared and kept at 4°C. These stock solutions are then mixed (referred to as the working solutions) for use on the day of testing. We have experimented with a freeze-dried working solution of mixed reagents and wish to report the feasibility of using this variation

MATERIALS AND METHODS

Preparation of reagents

Reagents were prepared according to the modified method of Beutler and Mitchell:³

Buffer - 0.2M TRIS, pH 7.8	
Beta-Nicotinamide Adenine	7.5 mM
Dinucleotide Phosphate (NADP)	
Glutathione-oxidised form (GSSG)	8.0 mM
Glucose-6-Phosphate disodium salt	10.0 mM

Procedure

1. Equal volumes of each reagent were mixed together.
2. Sodium azide was weighed and added to the mixture to a final concentration of 0.1%.
3. The resultant mixture was aliquoted in 100 ul volumes in 75 x 10 mm plastic tubes.

Freeze drying

1. The aliquots of mixed reagents were immersed in liquid nitrogen for at least 1 minute to ensure proper freezing.
2. The mouth of the plastic tube was covered with a parafilm and a few pin holes were made in the parafilm.
3. The plastic tube was then attached to the freeze dryer (Benchtop model - Virtis, USA) at -58°C, 0 millitorr, for at least 2 hours.

Reconstitution

Each tube was reconstituted in 100 ul of distilled water. The reconstituted mixture was used immediately.

G6PD screening test

21 blood samples, 12 with normal G6PD activity and 9 deficient in G6PD, collected in EDTA bottles were spotted on filter paper.

Additional negative controls using plasma and serum were also spotted on the filter paper. The following procedure was based on Beutler and Mitchell modified on the recommendation of the **ICSH**.⁴

1. A small disc of the dried sample of diameter 0.5 cm was punched from the filter paper.
2. The disc was then added to the reconstituted mixture and the tube was incubated at **37°C** for 10 minutes.
3. At the end of the **incubation**, a **small** sample of the reaction mixture was spotted onto a piece of **Whatman** filter paper using a capillary tubing.
4. The filter paper was then hot air-dried and viewed immediately under long wavelength UV light (366 nm).

The same samples were tested using the routine freshly made reagent mixture from stock solutions.

RESULTS

A positive reaction appeared as a brilliant blue spot under **W** illumination. **G6PD** deficient samples and negative controls did not show fluorescence. The normal samples (12) were all positive for **G6PD** and the deficient samples (9) showed no fluorescence. These results were similar to that of the freshly prepared reagent mixtures.

DISCUSSION

The freeze-dried mixture appeared as a white powder attached to the bottom of the plastic tube. The mixture dissolved in distilled water readily. It appeared that the process of freeze drying did not damage the activities of **NADP**

and **GSSG**. The reagent mixture did not give rise to false positive reactions and normal blood samples gave comparable fluorescence for both freeze-dried and freshly made reagent mixtures.

We are in the midst of conducting a full evaluation study including stability testing for the duration of storage and the effect of different temperatures on the **freeze-dried** material. We are of the opinion that freeze drying is a practical way to prepare reagent tubes in bulk. This also opens the way for a major laboratory to supply the reagents to smaller laboratories for screening of samples.

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REFERENCES

1. Phua KB. Glucose-6-phosphate dehydrogenase deficiency. Paediatric Update, 1989; 1: 2-3.
2. Dacie JV, Lewis SM. Practical Haematology. 6th ed. Edinburgh: Churchill Livingstone, 1984: 159-62.
3. Beutler E, Mitchell M. Special modification of the fluorescence screening method for glucose-6-phosphate dehydrogenase deficiency. Blood 1968; 32: 816-8.
4. Beutler E, Blume KG, Kaplan JC, Lohr GW, Romot B, Valentine WN. International Committee for standardization in haematology: recommended screening test for glucose 6-phosphate dehydrogenase (G-6-PD) deficiency. Brit J Haematol 1979; 43: 465.