A Stability Study of 6-Phosphogluconate Dehydrogenase Types in Bloodstains

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Abstract: The possibility of determination of 6-phosphogluconate dehydrogenase (PGD) types from bloodstains made on filter paper was examined by starch gel electrophoresis. Addition of NADP to the gel and cathodal buffers yielded a rather better visualization of isoenzyme bands. The time limits of determination of PGD types were: at 4°C 6 weeks, at room temperature 4 weeks, at 37°C less than 1 week.

Key words: Polymorphism, Stability study, PGD phenotypes, Grouping of bloodstains

INTRODUCTION

Polymorphism of human red cell 6-phosphogluconate dehydrogenase (PGD) was first discovered by Fildes and Part*). Using starch gel electrophoresis, they demonstrated three common phenotypes PGD A, PGD AC and PGD C determined by two codominant autosomal alleles PGD*A and PGD*C.

The PGD system is now an accepted genetic marker not only for paternity testing, but also for blood individualization¹⁴,6). However, there is a paucity of information on the determination limits at different temperature conditions under which the bloodstains are dried and stored, probably owing to its relatively unfavourable phenotypic distribution (own material: 84.7% PGD A, 14.3% PGD AC and 0.1% PGD C³¹).

This paper describes the results of a stability evaluation of PGD isoenzymes in bloodstains under various storage conditions.

MATERIALS AND METHODS

Venous blood from 20 donors with known phenotypes was dropped on filter paper (Toyoroshi No. 2, Tokyo) and allowed to dry for a few hours at room temperature. The bloodstains thus made were stored at 4°C in a refrigerator, at room temperature and at 37°C in a thermostatic chamber, and examined at 1-week intervals over a period of 10 weeks. The stains were cut in 3 x 8 mm pieces, moistened with 10 μl physiologic saline and directly inserted into the starch gel at the origin.

Horizontal starch gel electrophoresis was performed using 10% Starch Hydrolysed (Connaught Laboratories Limited, Ontario, Canada). The bridge buffer used was 0.1 M phosphate buffer (pH 7.0); the gel
buffer was prepared by diluting the bridge buffer 1:10. According to the suggestion of Ishimoto and Izumi\(^4\) that PGD isoenzymes in bloodstains can be clearly demonstrated with nicotinamide adenine dinucleotide phosphate (NADP), this coenzyme (2 mg/100 ml) was added to the gel and cathodal buffers. Electrophoresis was conducted at a constant voltage of 4 V/cm for 16 hours at 4°C. After the separation, the

Fig. 1. Electrophoretic patterns of PGD types in bloodstains stored at 37°C for 1 week (a), at room temperature for 2 weeks (b), at room temperature for 4 weeks (c) and at 4°C for 2 weeks (d). The anode is at the top.
gel was horizontally sliced and stained by the method of Fildes and Parr\textsuperscript{3}). The enzyme appeared as bright blue bands within 30 min.

**Results and Discussion**

The present study examined 20 bloodstains of which nine were PGD A, nine PGD AC and two PGD C. The types AC and C exhibited three isoenzyme bands with different intensities, while the type A showed one band corresponding in electrophoretic mobility with the most anodal band of type AC or C. The banding profile of types AC and C in bloodstains was generally indistinct as compared with that in hemolysates.

Addition of NADP yielded a rather better resolution of isoenzyme bands, which is consistent with the observation of Ishimoto and Izumi\textsuperscript{4}). Therefore, the use of NADP is recommended for the typing of PGD from bloodstains. It would appear that NADP plays a role in the maintenance of the structural integrity of the variant and normal PGD enzymes, as speculated by Davidson\textsuperscript{2}). To improve the electrophoretic separation of isoenzyme bands, we attempted experiments after pretreatment of the bloodstains with 0.03 M 2-mercaptoethanol or 0.05 M dithiothreitol. However, the results were unsatisfactory.

Fig. 1 shows the electrophoretic patterns of PGD types in bloodstains under different storage conditions. Table 1 summarizes the results for the time limits of determination of PGD types in bloodstains stored at 4°C, room temperature and 37°C. All the bloodstains examined were reliably typed for PGD at 4°C for periods of up to 6 weeks and at room temperature for periods of up to 4 weeks. The bands became fainter and more indistinct with the duration of storage, particularly in the type C. Our results obtained from bloodstains stored at room temperature are comparable to those reported by others\textsuperscript{1,4,6). Storage of bloodstains at 37°C gave much poorer results, and the typing was no longer successful after 2 weeks of storage.

In conclusion, the PGD typing is possible from bloodstains after fairly long storage periods (about 1 month at room temperature). This genetic marker is of practical use in medicolegal grouping of bloodstains, but seems of limited value in tropical regions.

**References**

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demonstration of three enzyme polymorphisms from bloodstains by simultaneous electrophoresis. Z. Rechtsmed., 69, 185–190, 1971.

血緑における6-フォスフォルコン酸脱水素酵素型の安定性

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抄 録：でんぶんゲル電気泳動法を用いて、蛍紙上に作成した血痕から6-フォスフォルコン酸脱水素酵素（PGD）型の検出を試みた。ゲルおよび陰極側電極槽用緩衝液にNADPを添加すると、酵素バンドは判定しやすくなった。PGD型の検出限界は4°Cで6週間まで、室温では4週間まで判定可能であったが、37°Cにおいては1週間ですでに判定できないものがあった。

キーワード 酵素多型、安定性試験、PGD型、血緑からの型判定