

A SIMPLE TECHNIQUE FOR THE DETERMINATION OF GGTP TYPES

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Introduction

Human seminal plasma contains a high enzyme activity of gamma-glutamyl transpeptidase (GGTP, EC 2.3.2.2) (Rosalki and Rowe 1973). Abe and Hiraiwa (1986) discovered polymorphism of seminal GGTP by means of slab-disc electrophoresis. We describe a simple procedure for phenotyping seminal GGTP using starch gel electrophoresis.

Materials and Methods

Ejaculates were obtained from 350 male volunteers living in Yamanashi Prefecture, a central part of Japan. Semen samples were absorbed onto 3 x 8 mm filter paper strips (Whatman No. 3, Maidstone, UK) and inserted in the slots of the gel 4 cm from the cathode.

Horizontal starch gel electrophoresis was performed by the method of Poulik (1957) using 10 % hydrolyzed starch (Biotest-AG, Frankfurt, FRG) on 150 x 150 x 4 mm gel plates. Electrophoresis was carried out at a constant current of 20 mA for 3.5 h at 4°C.

After run, the gel was horizontally sliced and covered with a 15 x 15 cm sheet of filter paper (Whatman No. 3) soaked in 15 ml 0.1 M glycylglycine containing 150 mg *L*-gamma-glutamyl-*p*-diethylaminoanilide dihydrochloride and 30 mg α -naphthol (prepare freshly and adjust to pH 8.6 with 2 N sodium hydroxide) for 3 h at 37°C. In order to avoid dessication the gel plate was wrapped with a sheet of thin plastic film. After incubation, the filter paper was removed and the gel treated with 0.2 % periodic acid for 5 min. The GGTP patterns appeared as blue bands.

Results and Discussion

Figure 1 shows three GGTP types (GGTP 1, 2-1 and 2) obtained by the present starch gel electrophoretic technique. GGTP 1 and GGTP 2 exhibited one major band with different electrophoretic mobility, each sometimes being accompanied with an additional minor band. GGTP 2-1 consisted of two bands: anodal one corresponded to the GGTP 1 band and cathodal one to the GGTP 2 band.

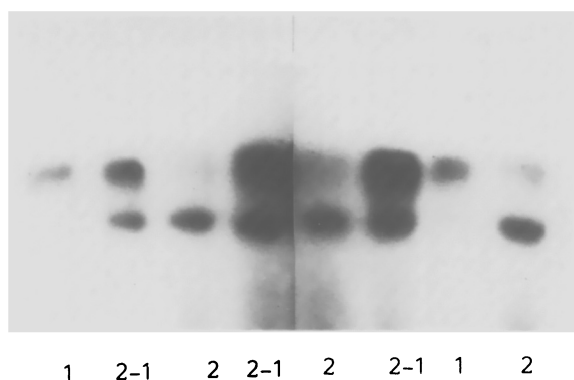


Fig. 1. Electrophoretic pattern of seminal GGTP types.
The anode is at the top

The results for the distribution are given in Table 1. The population data fitted the Hardy-Weinberg law. The GGTP*1 frequency in our population sample (0.616) is considerably high as compared with that observed by Abe and Hiraiwa (0.446).

Table 1. Distribution of GGTP types in a Japanese population

Phenotype	No. observed	(%)	No. expected
1	129	(36.9)	132.8
2-1	173	(49.4)	165.5
2	48	(13.7)	51.6
Total	350	(100.0)	350.0

Allele frequencies: GGTP*1 = 0.616, GGTP*2 = 0.384
 $\chi^2 = 0.73$, d.f. = 1, $0.50 > P > 0.30$

The present method is simple and rapid and will therefore have broad application to further forensic, anthropological and population studies.

References

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- Poulik MD (1957) Starch gel electrophoresis in a discontinuous system of buffers. *Nature* 180: 1477-1479