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BRIEF COMMUNICATIONS

Agarose Gel Electrophoretic Technique for Typing Peptidase A

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ABSTRACT An agarose gel electrophoretic technique for the typing of erythrocyte and seminal peptidase A enzyme has been reported.

INTRODUCTION

The presence of peptidase A (Pep A) in human semen (Blake and Sensabaugh, 1976, 1978) and its absence in vaginal fluid (Parkin, 1981; Divall, 1984) makes this enzyme a valuable genetic marker for identifying semen in sexual offence cases against women, especially when vasectomized or azoospermic persons are involved. In this paper, an agarose gel electrophoretic technique using a "continuous" buffer system has been reported. The use of the Indian snake (Russell's viper) venom in staining mixture in place of venom from American Crotalus admanteus has been found to be equally effective. The technique is comparatively simple and cheap than those reported earlier (Lewis and Harris, 1867, 1969; Blake et al., 1970, 1987; Rapley et al., 1971; Povey et al., 1972; Blake and Sansabaugh, 1976, 1978; Parkin, 1978, 1981; Divall, 1984).

MATERIAL AND METHODS

Samples: The samples analysed were haemolysates, liquid semen, semen stains and vaginal swabs with or without semen.

Buffers: Bridge: 0.1 M Maleic acid added to 0.1 M Tris untill pH 7.4

Gel: 1.10 dulution of bridge buffer

Gel Preparartion and Sample Application: 30 ml of 1% Agarose (Litex HSIF) dissolved in gel buffer were used for casting a 15x11 cm gel on a thin glass plate. Slots were made along the width of the gel using 6 mm broad Whatman No.3 filter paper strips, 3 cm from the cathodal edge. These were filled with different samples of haemolysates, extract of semen stains and vaginal swabs. Small pieces of cotton wool from the vaginal swabs, moistened with gel buffer were also placed in some of the slots.

Electrophoresis: Electrophoresis was carried out at 300 V (Approx. 11mA) for 1.5 -2 h on a cooling plate through which water at 9° C was circulated. The wicks used consisted of double layer of Whatman No.3 filter paper.

Staining Mixture:

0.05 M Tris buffer pH 8	15 ml
L-valyl - L- leucine	5 mg
L-amino acid oxidase (Indian Russell's viper venom, Haffkine I.T.R.T., Bombay)	5 mg
MTT	2 mg
PMS	1 mg
Agar	150 mg

Development of Pep A Bands: Staining mixture was poured between origin and anodal edge and the gel was incubated in moist chamber at 37°C for at least 1 h for complete development of the bands.

RESULTS AND DISCUSSION

This technique was applied to identify Pep A in over 80 haemolysates and 10 semen samples,

and in 130 vaginal swabs (post coital and semen free). In all the blood and semen samples and in vaginal swabs containing semen, Pep A activity appeared as a single band at the same level around 6 cm from the origin. No such band appeared in semen-free vaginal swabs.

In some vaginal swabs (with or without semen) one or two faster bands appeared at different levels between this band level and anodal edge of the gel. These additional bands correspond to the bacterial peptidase bands of Blake et al. (1987). A slow band of peptidase S also appeared in 3 of the post-coital vaginal swabs.

The present technique being easier, faster and cheaper, is beings applied to the detailed study of Pep A activity in post-coital vaginal swabs of different time intervals as well as in samples of blood and semen of differents ages. The preliminary results show the detectability of this enzyme in vaginal swabs taken after 15 hours of intercourse.

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