Isolation and Identification of ABO Blood Group of Seminal Stains Mixed with Vaginal Secretion or Blood

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The identification of seminal stains contaminated with body fluid is one of the most difficult problems in forensic medical biology. The methods such as electrophoretic separation, ABO blood typing of sperm in mixed stains and absorption-inhibition test etc, have been used for this purpose. This paper reports a new method for the ABO blood typing in seminal stains mixed with vaginal secretion or blood using nitro cellulose filter (NCF) coated with anti-semino peculiar proteins (ASPP).

Basic Procedures

1. Preparation of immunogen and immunization of rabbits

The semens obtained from a number of donors were mixed and the mixture was centrifuged. The supernatant was isolated by 1% agarose gel electrophoresis (pH 8.6, barbital buffer, 320V, 3 - 4 hrs, 10 C). The gel containing a large amount of seminoglycoproteins was cut off 5-6 cm far from origin to anode. Then the gel was frozen, thawed and extracted by centrifugation. The supernatant was concentrated into a solution of 1mg protein/ml and then used as immunogen. Crude antiserum was prepared by immunizing rabbits. Rabbits were intradermally injected with immunogens in the presence of Freund's adjuvant at intervals of 10, 15 and 30 days. When antibody titer reached 5000 fold, the blood was taken from rabbits and used as crude antiserum.

2. Purification of crude antiserum

The crude antiserum which had previously been absorbed with A,B,O type red cells was purified by affinity chromatography using CNBr - activated sepharose 4B. The human serum and vaginal secretion were used for coupling respectively. In coupling procedure, the human serum was diluted with 0.1 M NaH-CO3 (pH 8.4) into a solution of 10-15mg proteins/ml (pH 7.8). The vaginal secretion was extracted with saline and then centrifuged. The supernatant was dialyzed in saline for 48hrs and concentrated into a solution of 3mg protein/ml (pH 7).

3. ABO blood typing in seminal stains mixed with vaginal secretion or blood by NCF.

A piece of NCF (5 x 5mm) was put in the ASPP, absorbed in vacuum (-760mmHg) for 20 minutes at room temperature and washed in cold saline for removing the unabsorbed serum proteins.

The NCF coated with ASPP was put in the mixture of seminal-vaginal stains or seminal-blood stains, absorbed in vacuum (-760mmHg)for 10 minutes at room temperature and washed in cold saline for removing unreacted proteins.

Then the NCF was put in a well of the glass plate and one drop of saline was added to it. The substance specifically reacted with the antibody was eluted from the reactant on the NCF at 56C for 7 minutes. A stripe of cotton gauze (1cm in length) was immersed in the eluate and dried. Finally, ABO blood typing of seminal stains was determined by the egg white absorption-elution method.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr
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Seminal protein may contain two antigenic determinants: the peculiar proteins and ABO blood group. The ASPP reacts with seminal peculiar protein (SPP) and forms a reactive complex, in which the ABO blood group antigen determinant is present. After the NCF coated with ASPP is washed, only the SPP-ASPP reactive complex remains on the NCF. Hence the proteins in eluate contains ABO blood group antigenic determinant too. Then ABO blood typing could be determined by the egg white absorption-elution method.

The results indicate that the new method mentioned above is accurate, simple, time saving and sensitive.

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