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**APPLICATION OF PHYTOAGGLUTININS FOR DETERMINING THE
GROUP AFFILIATION OF SEMEN BY THE ABSORPTION–ELUTION
METHOD IN FORENSIC MEDICAL PRACTICE**

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INTRODUCTION

Forensic medical examinations assist judicial and investigative authorities in reconstructing the circumstances of a crime. In particular, they can substantially narrow the range of suspected individuals and, in some cases, provide objective evidence that either supports or excludes the involvement of a particular suspect. These examinations include the investigation of stains of biological secretions found on physical evidence. In particular, such examinations make it possible to determine the species origin and immunoserological characteristics (group antigens) of biological secretions, as well as to assess whether a stain of a particular secretion could have originated from a specific person (suspect or victim) or whether that person can be conclusively excluded. Law enforcement agencies frequently detect blood, semen, and similar biological stains at crime scenes and collect them as physical evidence. The presence of a particular biological secretion on physical evidence may indicate the nature of the crime committed. For example, the detection of semen stains on clothing or bed linen may be relevant in cases of sexual assault, sexual intercourse with minors, and other offenses.

Determination of the group affiliation of such stains also makes it possible to compare the group characteristics of the biological secretion with those of a suspect or accused person. Under modern conditions, the rapid and comprehensive investigation of many crimes depends on the application of the latest scientific advances. It is necessary to examine mixed blood and semen stains on physical evidence exposed to various environmental factors, as well as stains of blood and semen of different ages, and to determine their group affiliation. In many cases, semen must be identified in stains formed by mixtures of different biological secretions. In such situations, the characteristics of spermatozoa make it possible to establish the presence of semen and identify specific characteristics, for example, when interpreting findings in mixed vaginal smears. It is essential for a forensic medical expert to be able to reliably determine the presence, type, and group affiliation of biological secretions of the human body on physical evidence; to examine biological secretions in a pure, uncontaminated state, as well as in mixtures with blood or with one another; and to differentiate one biological secretion from another. Determining whether mixed blood and semen originate from a particular individual remains one of the important problems in forensic medical examination.

Aim of the Study. The aim of the study was to determine A, B, and O(H) antigens in semen stains on physical evidence using the absorption–elution method.



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Materials and Methods. The study materials consisted of 42 experimental semen stains and stains from 35 items of forensic examination material obtained from the forensic biology laboratory of the Surkhandarya Branch of the Republican Scientific and Practical Center of Forensic Medical Examination during 2017–2020.

Results. The absorption–elution method is used in the forensic medical laboratory for the examination of semen. To determine the immunoserological group affiliation of semen according to the AB0 system, the absorption–elution method was applied using anti-A phytoagglutinin (“Nimrang” - grape extract), anti-B phytoagglutinin (*Sophora japonica* L.-sophora extract), and anti-H phytoagglutinin (*Sambucus ebulus* L. - elderberry extract). For this purpose, 12 semen samples obtained from men aged 25–45 years were examined. For the analysis, a piece of the examined semen stain together with its carrier material, as well as control semen samples of known groups 0 $\alpha\beta$ (I), A β (II), B α (III), and AB0(IV) and control clean gauze, were cut into pieces measuring 0,5–0,6 cm. The samples were placed separately into heat-resistant glass containers, and 10% sodium hydroxide solution at pH 7.4 was added. The samples were then boiled for 10 minutes. After drying on filter paper, the samples were placed separately into specially labeled laboratory containers. Extracts of anti-A phytoagglutinin “FAa-A” at a titer of 1:64, anti-B phytoagglutinin “FAa-B” at a titer of 1:128, and anti-H phytoagglutinin “FAa-H” at a titer of 1:64 were added. The samples were placed in a refrigerator at +4 to +6°C for 20-24 hours for absorption. The threads were then placed into a special container with wells and washed 3-4 times with ice-cold physiological saline. The washed threads were dried on filter paper and separately placed into specially labeled laboratory containers. Two drops of physiological saline were added to each sample, and the test-tube rack was placed in a thermostat at +52°C for 30 minutes. One drop of 1% A, B, and O erythrocyte suspensions was added to the test tubes labeled K, 0 $\alpha\beta$ (I), A β (II), B α (III), AB(IV), “FAa-A”, “FAa-B”, and “FAa-H”, followed by centrifugation at 1,500 rpm for 4 minutes. One drop from each test tube was then transferred onto a glass slide, covered with a coverslip, and examined microscopically.

No agglutination was observed in the control clean gauze samples with the known semen samples of groups 0 $\alpha\beta$ (I), A β (II), B α (III), and AB(IV). The known 0 $\alpha\beta$ (I) semen sample showed an agglutination reaction only with anti-H phytoagglutinin, indicating the presence of the 0(H) antigen. The known A β (II) semen sample showed an agglutination reaction only with anti-A phytoagglutinin, indicating the presence of the A antigen. The known B α (III) semen sample showed an agglutination reaction only with anti-B phytoagglutinin, indicating the presence of the B antigen.

The known AB(IV) semen sample demonstrated agglutination reactions with both anti-A and anti-B phytoagglutinins, indicating the presence of A and B antigens. Among the study samples, objects No.1,4,5, and 12 demonstrated an agglutination reaction only with anti-B phytoagglutinin, indicating the presence of the B antigen. Therefore, these stains could have originated from the semen of men belonging to blood group B α (III). In objects No. 2, 6, and 8, an agglutination reaction was observed only with anti-H phytoagglutinin, indicating the presence of the O(H) antigen. Thus, these stains could have originated from the semen of men belonging to blood group 0 $\alpha\beta$ (I). In objects No. 3, 7, and 9, an agglutination reaction



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was observed only with anti-A phytoagglutinin, indicating the presence of the A antigen. Therefore, these stains could have originated from the semen of men belonging to blood group A β (II). In objects No. 10 and 11, agglutination reactions were observed with both anti-A and anti-B phytoagglutinins, indicating the presence of A and B antigens. Therefore, these stains could have originated from the semen of men belonging to blood group AB(IV).

Conclusion. It has been demonstrated that phytoagglutinins - extracts of *Sophora japonica* L., “Nimrang” grape seeds, and *Sambucus ebulus* L. - can be used in expert forensic practice for the examination of physical evidence and for the detection of A, B, and 0(H) antigens in semen and blood stains using affinity chromatography, a quantitative modification of agglutinin absorption, and the absorption-elution method. Thus, the obtained findings demonstrate the feasibility of using anti-A phytoagglutinin (“Nimrang” grape extract), anti-B phytoagglutinin (*Sophora japonica* L. *sophora* extract), and anti-H phytoagglutinin (*Sambucus ebulus* L. elderberry extract) to determine A, B, and 0(H) antigens in semen stains located on various carrier materials.

