

DETERMINING SPECIES OF BLOOD USING THE RADIAL IMMUNODIFFUSION METHOD IN AN ANTIBODY-GEL MATRIX

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Relevance. In forensic practice, the identification of biological evidence, particularly blood traces, and the determination of their species affiliation is of significant diagnostic importance. Experience shows that identifying small, faint, long-stored, or altered blood traces resulting from external factors (washing, chemical exposure, contamination) often presents challenges when using traditional serological methods. Despite the widespread use of the precipitation reaction in a liquid medium, the Ouchterlony method, and immunoelectrophoresis, their sensitivity is not always sufficient. Therefore, there is a growing need for highly sensitive, specific, and simple-to-perform methods.

Research Objective. The objective of this research is to study the potential of the radial immunodiffusion method in an antibody-gel matrix for determining the species of blood traces in forensic practice, to evaluate its sensitivity and specificity, and to develop optimal conditions for conducting the reaction.

Scientific Basis and Theoretical Aspects. The radial immunodiffusion method was first proposed by G. Petrie and later developed by G. Mancini and his colleagues. The method is based on the diffusion between an antigen and an antibody. The antibody is distributed uniformly within the gel medium, while the antigen diffuses radially from a specially made well. A precipitation ring is formed when they meet at optimal concentrations.

The diameter of the precipitin ring is directly proportional to the antigen concentration and inversely related to the antibody concentration. The optimal ratio between antigen and antibody (1:20 - 1:100) ensures the accuracy and sensitivity of the reaction. A key aspect of this method is that it allows for not only qualitative but also quantitative assessment.

Materials and Methods. The subjects of the study were human and various animal (horse, cow, pig, dog, cat, chicken) blood sera, as well as experimental bloodstains. To separate the serum from blood samples, they were incubated at 37°C and then centrifuged at 3000 rpm for 15 minutes. Experimental bloodstains were prepared on filter paper and various textile materials. The samples were stored for periods ranging

from 2 days to 6 months and were studied under various conditions (washed, contaminated, exposed to chemical agents).

A total of 107 blood serum samples and 238 bloodstains were examined. Diagnostic precipitating sera with a high titer (at least 1:10000) were used in the reaction. The antibody-gel matrix was prepared using an agar and veronal-acetate buffer solution. The gel was poured onto glass slides, special wells were created, and antigens were introduced into them. The reaction was conducted in a humid chamber at room temperature.

Results. The research findings indicated that the radial immunodiffusion method possesses high sensitivity and specificity. In the presence of homologous antigens, distinct and clearly visible precipitation rings were formed.

Using this method, the following were successfully identified:

- new and old bloodstains,
- minute biological traces,
- washed and chemically treated samples,
- blood traces absorbed into various materials.

Precipitation rings were not observed in control samples and with heterologous antigens, which confirms the high degree of specificity of the method. Furthermore, the reaction results were more clearly visualized through staining.

Discussion. The obtained results demonstrate the superiority of the radial immunodiffusion method over traditional serological methods. The method enables the detection of small-volume and degraded blood traces. Moreover, its simplicity, low cost, and the fact that it does not require specialized, complex equipment allow for its widespread practical application.

However, the effectiveness of the method depends on the antibody and antigen concentrations, the gel composition, and the reaction conditions, making the standardization of these factors crucial. The method allows for the detection of small-volume and degraded blood traces.

Conclusion. The radial immunodiffusion method using an antibody-gel matrix is a highly effective, sensitive, and reliable method for determining the species of origin of blood in forensic practice. This method is particularly effective in identifying complex, old, or trace amounts of biological evidence, and its widespread implementation in forensic medical examinations is recommended.

The radial immunodiffusion method on the antibody-gel matrix is a sensitive and reliable method for determining blood type in forensic medical practice. This method is especially effective in identifying complex, old, or small amounts of biological traces, and it is advisable to widely implement it in forensic medical examination.

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