

UDK:619:616.9:636.3:616.098

**BIOTECHNOLOGICAL PROCESSING OF HEMOLYSIS FROM BLOOD
SAMPLES OBTAINED FROM BLOOD SAMPLES.**

Rajabova Munira Akhmad kizi

Master

Aliyev Dilmurod

Doctor of Biological Sciences, Associate Professor
Samarkand State University of Veterinary Medicine,

Animal Husbandry and Biotechnology

<https://doi.org/10.5281/zenodo.22141615>

Annation: In the process of preparing hemolysin from blood samples, biological materials were collected in accordance with veterinary-serological requirements and carried out in laboratory conditions. The steps of obtaining hemolysin were carried out by isolating erythrocytes from blood samples and immunobiologically processing them.

Key words: Blood, hemolysin, complement binding reaction, sheep, bacteria, PBS, erythrocyte, plasma.

Relevance of the topic. Currently, one of the main fundamental areas of modern microbiology and medicine in the world and in our Republic is the study of microbial pathogenicity. It is known that pathogenic bacteria produce various substances that directly damage or kill macro and microorganism cells, as well as facilitate their entry into the body by overcoming the defense systems of bacteria. These substances play a major role in the development of diseases caused by bacteria.

Hemolysins are one of the biological substances that actively participate in immunological reactions, they have the property of ensuring the destruction of erythrocytes. Hemolysin complexes are widely used in the complement fixation reaction, hemolytic tests and other serological tests. The quality of these preparations is one of the main factors determining the accuracy and reliability of diagnostic tests. Therefore, improving the technology for preparing hemolysin complexes, increasing their biological activity and standardization is of particular importance today.

According to the current scientific literature, bacterial toxins are a group of bacterial products that have the ability to harm or kill living organisms when exposed to relatively small amounts. Today, it is difficult to give a precise definition due to the diversity of bacterial toxins in their structure, mechanisms of action, immunogenicity, and other properties. As a rule, toxins are relatively high-molecular substances in the form of proteins, peptides, or polysaccharides. However, some bacterial toxins with molecular weights of less than 10,000 Da are also known [1; 23-28 p., 9; 114-119 p.].

One of the most important questions in the study of toxins is the question of their role in pathogenesis. This question is most easily answered in cases where the disease is a direct consequence of intoxication, such as food poisoning. In such cases, living bacterial cells are often destroyed when exposed to the toxic substance, and experimental reproduction of the disease can occur as a result of direct exposure to the toxin. When the production of this substance is carried out by the multiplication of the bacteria in the host organism, it is somewhat more difficult to determine the role of the toxin in pathogenesis. In such cases, a number of conditions are necessary for the "successful" production of the toxin. Such conditions include additional factors produced by the microbe, which are necessary for its entry into and maintenance in the host organism. When the bacterium multiplies in the host organism, it is

Scottish International Conference on Multidisciplinary Research and Innovation – SICMRI 2026

much more difficult to assign a specific role to a particular toxin. Nevertheless, it can be argued that all diseases caused by bacteria are directly or indirectly caused by the action of toxins [2; pp. 1011-1018, 3; pp. 125-130, 4; pp. 238-241].5; pp. 21-35, 6; pp. 70, 7; pp. 39-64, 8; pp. 75-89].

Hemolysin is a special immunological substance that has the property of dissolving erythrocytes. It is mainly used in complement fixation reactions (CFR), hemolytic reactions and serological laboratory tests. Hemolysin is a biologically active reagent that acts on the erythrocyte membrane and causes cell lysis.

In this experiment, 10 sheep blood samples taken from the “Zayniyeva Zamira Safarovna” farm were biotechnologically processed and hemolysin activity was determined.

In our research, we carried out work on obtaining high-quality hemolysin from sheep blood and determining the biological activity of hemolysin, assessing its suitability for use in the complement fixation reaction, and analyzing the hemolytic activity of each sample.



LABORATORY WORKING PROCESS PICTURES

Our research was carried out using sterile syringes and needles, test tubes, centrifuge, thermostat (37°C), physiological solution (0.85% NaCl), sheep erythrocytes, hemolytic serum, complement, micropipette, sterile filter, laboratory stand.

When collecting blood samples, 5–10 ml of venous blood was collected under sterile conditions from 10 healthy sheep on the farm. Blood was collected in special sterile test tubes. During blood collection, aseptic rules were strictly observed, the samples were numbered, and delivered to the laboratory.

For serum extraction, blood samples were centrifuged at 3000 rpm for 10 minutes. As a result, serum was formed in the upper layer and erythrocyte mass in the lower layer. Serum was transferred to separate test tubes using a sterile pipette.



LABORATORY WORKING PROCESS PICTURES

Scottish International Conference on Multidisciplinary Research and Innovation – SICMRI 2026

During erythrocyte washing, the erythrocytes isolated were washed 3 times with physiological solution, excess protein and plasma residues were removed, erythrocyte purification, and hemolytic reaction were studied.

Hemolysin formation: hemolytic serum was added to the washed erythrocytes and the mixture was incubated at 37°C for 30–60 minutes. In this process: antibodies against erythrocytes were combined; an immunological complex was formed; a hemolysis reaction occurred.

Biotechnological processing The following treatments were performed on the obtained hemolysin.

During filtration, the mixture was passed through a sterile filter. During standardization, the hemolysin titer was determined by laboratory methods. Processing was carried out in accordance with the requirements of sterilization and biological safety. The finished hemolysin was stored at a temperature of +4°C.

Results from 10 sheep samples at the Zayniyeva Zamira Safarovna farm

T/r	Sheep number	Hemolysis Level	Hemolysin titer	Lab Score
1	Sheep-1	Severe	1:1600	Active
2	Sheep-2	Moderate	1:800	Good
3	Sheep-3	Severe	1:1600	Active
4	Sheep-4	Low	1:400	Weak
5	Sheep-5	Very Severe	1:3200	Very active
6	Sheep-6	Moderate	1:800	Good
7	Sheep-7	Low	1:200	Weak
8	Sheep-8	Severe	1:1600	Active
9	Sheep-9	Moderate	1:800	Good
10	Sheep-10	Very strong	1:3200	Very active

In our experiments, according to Table 1, active erythrocyte fragmentation was observed in the sample taken from the first sheep. The hemolysin titer was high and was considered suitable for laboratory diagnostics.

In the second sheep, hemolytic activity was moderate. It reacted well with complement.

In the third sheep, hemolysis was clearly and completely observed. It was noted that biological activity was high.

In the fourth sheep, hemolysis was slow. It was assumed that the concentration of antibodies against erythrocytes was low.

In the fifth sheep, the highest biological activity was recorded. The hemolysin titer was very high and was evaluated as a standard diagnostic reagent.

In the sixth sheep, the hemolytic reaction was stable. A result was obtained that meets laboratory requirements.

In the seventh sheep, hemolysis was weak. Due to the low titer, additional processing was recommended.

In the eighth sheep, strong erythrocyte lysis was observed. Hemolysin was considered highly active.

In the ninth sheep, moderate hemolytic activity was detected. Suitable for use in serological tests. In the tenth sheep, very high hemolytic activity was observed. It was noted as one of the best results.

Scottish International Conference on Multidisciplinary Research and Innovation – SICMRI 2026

During the experiment, hemolysin was successfully obtained from 10 sheep blood samples. Among the samples, the highest hemolytic activity was detected in sheep 5 and 10. Samples from sheep 4 and 7 showed a weak reaction. As a result of biotechnological processing, the biological activity of hemolysin was preserved, a standard reagent was prepared for the laboratory, and the possibility of using it in the complement fixation reaction was created.

In conclusion, the technology for obtaining hemolysin from sheep blood samples taken from the farm "Zayniyeva Zamira Safarovna" was successfully implemented. As a result of biotechnological processing, high-quality and active hemolysin was obtained. The resulting preparations were found to be suitable for use in serological diagnostics and complement fixation reactions.

References

1. Баранцевич, Е.П. Применение MALDI-TOF масс-спектрометрии в клинической микробиологии //Трансляционная медицина. - 2014. - № 3. - С.23-28.
2. Баранов, А.А. Новая эпоха в медицинской микробиологии / // Вестник РАН. - 2015. - Т.85. - №11. - С.1011-1018
3. Бахир, В.М. Микробные биопленки: механизмы образования и взаимодействия с окружающей средой, новые подходы к разрушению биопленок - // Матер. XIII междунаро. науч.-практ. конф. «Пища. Экология. Качество», Красноярск. - 2016. - С.125-130.
4. Титова, С.В. Моделирование биопленок холерного вибриона на твердых поверхностях (стекло и пластик) и визуализация их в световом и люминисцентном микроскопах // Клиническая и лабораторная диагностика. - 2016. - Т. 61. - № 4.- С.238-241.
5. Дубровский, А.Я. Определение токсинов пептидной природы методом MALDI-MS (обзор)// Научное приборостроение. -2010. - № 4. -С.21-35.
6. Ильина, Е.Н. Молекулярные средства измерения в современной микробиологической лаборатории // Клин. лаб. диагностика. -2013. - № 9. - С.70.
7. Gu L. Q., Dalia S. M., Vincent J. B., Vigh G., Cheley S., Braha O., and Bayley H. (2000) Reversal of charge selectivity in transmembrane protein pores by using noncovalent molecular adapters. Proc Natl Acad Sci, N. 97, P. 3959-3964.
8. Hertle R. (2000) Serratia type pore forming toxins. Curr. Protein Pept. Sci., N. 1, P. 75-89.
9. Германчук, В.Г. Применение методов спектроскопического анализа для выявления холерного токсина // Здоровье населения и среда обитания. - 2016. - № 6 (279). - С.40-43.