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# Local Hemolysin and Complement Results Of Laboratory Testing for the Diagnostic of Animal Brucellosis

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***Abstract.*** *The article presents the findings of a comparison between hemolysin and complement, which are necessary for RBT in the diagnosis of brucellosis in animals raised in the brucellosis laboratory of the Research Institute of Veterinary Medicine, and their comparison with laboratory equivalents.*

***Key words:*** *brucellosis, RBT, AR, KBR, component, titer, hemolytic zardob, suspension, hemolysis, analogue, reagent.*

## Introduction

A persistent zoonotic illness that affects both humans and animals, brucellosis remains a major issue in both veterinary and contemporary medicine. The illness harms cattle greatly economically and is quite common around the world.

In the battle against brucellosis, successful outcomes are mostly determined by how well diagnostic tests work. Laboratory diagnosis are based on serological research. Today, the Rose Bengal test (RBT), agglutination reaction (AR), and complement binding reactions (KBR) are specifically utilized as serological assays.

These days, the majority of tests used to diagnose animal brucellosis are imported from overseas. In particular, the Republic of Kazakhstan and the Russian Federation are the primary importers of the component-complement utilized in CBR. Based on the aforementioned, the VITI Brucellosis laboratory generated an experimental series of hemolysin and complements utilized for the complement binding reaction (CBR).

**Objectives** - In order to perform the Complement Binding Reaction (CBR), an experimental series of hemolysin and complements generated in the Veterinary Research Institute's Brucellosis Laboratory are checked using blood sera obtained from farms with various brucellosis epizootic situations and compared with analog components.

**Methodology**. The approach of V.S. Kalinin and S.I. Ginzburg, as well as distinct stages of the existing technology of the Kursk biofactory, were followed in the development of hemolysin production and technological parameters. Utilizing the knowledge and equipment from the previous Alma-Ata Biofactory, supplement was produced.

The Brucellosis laboratory, current manufacturing circumstances, and locally available raw materials were utilized to enhance the hemolysin and complement production technology parameters.

Ram erythrocytes were administered intravenously in five separate treatments, separated by two days, to four rabbits weighing between three and five kilograms. The injections ranged from 0.25

to 1.25 milliliters, and the rabbits were bled seven days following the final injection. In order to eliminate rabbit complement, the acquired serum was inactivated for 30 minutes at a temperature of 56–58°C. The resulting serum (hemolysin) is divided into two parts and placed into 20 cm3 vials, with one portion preserved with glycerol in a 1:1 ratio and the other part with 0.5% phenol.

Using different nutrients created in accordance with the current formula that satisfies GOST 20730 standards, the resultant serum was tested for sterility.

As diluents, sterile chemically pure glycerol solution and normal bovine blood serum were employed.

100% hemolysis of ram erythrocytes in the hemolytic system was used to measure the hemolytic serum activity. Hemolysin was titrated at a 1:20 dilution with complement in the interim. The lowest amount (i.e., maximum dilution) of hemolytic serum, or 0.5 $cm^3$ of a 2.5% erythrocyte suspension in a total volume of 2.5 $cm^3$ of liquid, was determined to be the titer. This amount was then diluted with 0.5 $cm^3$ of complement to get a 1:20 ratio.

The total lack of hemolysin in ram erythrocytes without complement indicates the harmlessness of hemolytic serum.

**Result and discussion.** After the addition of glycerol, the hemolytic serum from all four experimental samples was combined into a single volume, and titration of the resultant serum showed the following: For 10 minutes, 0.5 ml of a 2.5% erythrocyte suspension and 0.5 ml of diluted 1:20 complement led to full hemolysis; the maximum hemolysin small titer was discovered to be 1:2000 (nominal titer). On the other hand, hemolysis was absent from every tube used as a negative control. Following this, 1:1500 and 1:1750 titers of hemolytic sera were generated by completely diluting with normal rabbit serum. Table 1 displays the hemolysins' comparative titration findings.

Table 1

COMPARATIVE TITRATION RESULTS OF HEMOLYSINS FROM DIFFERENT MANUFACTURERS

| Components | Dilution of hemolysin | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1:500 | 1:1000 | 1:1500 | 1:2000 | 1:2500 | 1:3000 | 1:3500 |
| VITI hemolysis, UzR | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 |
| "Shelkovo" hemolysis, RF | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 |
| Complement (1:20) | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 |
| Erythrocytes (2.5% suspension) | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 |
| Phys. Solution | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Water bath at a temperature of 37-38°C for 10 minutes | | | | | | | |
| The minimum value allowed | **CH** | **CH** | PH | PH | PH | PH | PH |
| VITI hemolysis, UzR | CH | CH | CH | **CH** | PH | PH | PH |
| "Shelkovo" hemolysis, RF | CH | CH | CH | CH | **CH** | PH | PH |

*Note: CH - complete hemolysis; PH – partial hemolysis*

The findings of the hemolysin titration at 1:1500 and 1:1750 were found to be comparable to those of the hemolysin generated in Russia at these same titration ratios. This suggests that hemolysin may be manufactured commercially and in a lab using domestic raw ingredients in our Republic.

In contrast to the analogue reagent made in Russia, Table 2 displays the titration findings of the hemolytic system of complement acquired from four guinea pigs at the VITI Brucellosis Laboratory. In addition, at a temperature of 38°C in water that was +10°C, the titer of complements in the hemolytic system was thought to be the minimum quantity that completely hemolyzed erythrocytes.

**Table 2**

**Results of comparative titration of "VITI" and "Shyolkovo biofactory" complements in the hemolytic system**

| Components | Test tube numbers | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| VITI complement, UzR (1:20) | 0,02 | 0,04 | 0,06 | **0,08** | 0,10 | 0,12 | 0,14 | 0,16 | 0,18 | 0,20 |
| "Shelkovo" complement, RF (1:20) | 0,02 | 0,04 | 0,06 | **0,08** | 0,10 | 0,12 | 0,14 | 0,16 | 0,18 | 0,20 |
| Physiological solution | 0,18 | 0,16 | 0,14 | 0,12 | 0,10 | 0,08 | 0,06 | 0,04 | 0,02 | - |
| Doubled titer hemolysin | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 |
| 2.5% ram erythrocyte suspension | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 |
| Physiological solution | 0,4 | 0,4 | 0,4 | 0,4 | 0,4 | 0,4 | 0,4 | 0,4 | 0,4 | 0,4 |
| Water bath at a temperature of 37-38°C for 10 minutes | | | | | | | | | | |
| The minimum value allowed | CH | CH | CH | **PH** | PH | PH | PH | PH | PH | PH |
| VITI complement, UzR (1:20) | CH | CH | CH | **PH** | PH | PH | PH | PH | PH | PH |
| "Shelkovo" complement, RF (1:20) | CH | CH | CH | **PH** | PH | PH | PH | PH | PH | PH |

*Note: CH - complete hemolysis; PH – partial hemolysis*

Table 2 shows that the titer of all compared complements in the hemolytic system was 0.08, meaning that it made no difference. The smallest amount of complements, when diluted in a ratio of

1:20, caused complete hemolysis of erythrocytes for 10 minutes in a water bath at a temperature of +37+38°C.

**Conclusion.** As a result, tests on local hemolysin and complements meant to diagnose brucellosis failed to find a discernible difference in their activity. This suggests that our nation has the capacity and necessity to produce these reagents on an industrial and laboratory scale. Furthermore, the development and use of local hemolysin and complement broadens the field of research of infectious illnesses in animals, including mange, leptospirosis, trypanosomosis, toxoplasmosis, salmonellosis, and other bacterial and viral infections that are utilized in CBR diagnosis.

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