METHODS OF DETECTION OF SPECIFIC ANTIBODIES IN BRUCELLOSIS

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Plate Agglutination Reaction (Heddleston Reaction). The advantage of this method is the simplicity of setting up the reaction, quick results, and the sensitivity of the reaction. A single brucellosis diagnosticum is used as an antigen to perform the Heddleston and Wright reactions. In the case of a positive reaction, flakes (agglutinate) appear in the drops of serum containing the antigen in the very first minutes. The maximum observation period is 8 minutes. For the diagnosis of brucellosis, only a positive result of the reaction is important. In cases of uncertain or negative results, along with epidemiological or epizootic indications, or in hospitals and donor screenings where it is necessary to determine the titer of agglutinins and their dynamics, the Wright, Coombs, Passive Hemagglutination Reaction (PHAR), and Enzyme-Linked Immunosorbent Assay (ELISA) should be used [1,4,6,8,9].

Tube Agglutination Reaction (Wright Reaction). The agglutination reaction is one of the main methods for diagnosing brucellosis in humans. It holds the most diagnostic value in acute and subacute forms of brucellosis. A diagnostic titer is considered to be an agglutination reaction of at least 2+ at a serum dilution of 1:100 or higher [7,8,9].

Antiglobulin Test (Coombs Reaction). In diagnosing brucellosis in humans and animals, especially with chronic infections where the agglutination reaction may be negative or positive at low titers, it is important to identify incomplete antibodies. The Coombs reaction uses a pre-titrated antiglobulin serum against human globulins (for example, antiglobulin serum for immunoelectrophoresis against human globulins). A diagnostic titer in the Coombs test is considered to be agglutination of at least 2+ at a serum dilution of 1:50 [1,2,3].

Passive Hemagglutination Reaction (PHAR). PHAR is a specific and highly sensitive method for detecting brucellosis antibodies in human serum. The serum titer is determined by the last dilution that yields a reaction of at least 3+. Interpretation of

PHAR results in humans: a titer of 1:50 is considered doubtful, while a titer of 1:100 and higher is considered positive [10,11,12].

Enzyme-Linked Immunosorbent Assay (ELISA). This method is used to diagnose all forms of the disease and during epidemiological screening of the population, as well as when selecting individuals for vaccination against brucellosis. A diagnostic ELISA test system is used to determine brucellosis antibodies. Specific antibodies in human serum are detected through the interaction of brucellosis antigen (LPS), adsorbed on a polystyrene flat-bottom plate, with the antibodies of the tested serum. A diagnostic titer in ELISA is considered a serum dilution of more than 1:400 [13,14,15].

Tests Revealing Increased Sensitization to Brucellosis Antigen.

Burnet Skin Allergy Test (Intradermal Allergy Test): This test is based on the ability of the body sensitized to brucellosis antigen to respond specifically with a local reaction (swelling, pain) to the intradermal administration of brucellosis allergen. The reaction is specific but appears in patients later than antibodies and persists for a long time, sometimes for years, after the clinical symptoms disappear. It should be noted that a positive allergic reaction may occur in cases of asymptomatic infection, as well as in individuals vaccinated with live brucellosis vaccine and those who have had long-term contact with the specific antigen [16,17,18].

Leukocyte Lysis Reaction. The introduction of a specific antigen into a sensitized organism is significant for the examined individual. Therefore, an effective method for detecting delayed hypersensitivity in vitro is the leukocyte lysis reaction (LLR). LLR is based on the registration of the destruction of leukocytes in a sensitized organism under the influence of a specific antigen, recorded in vitro. LLR has strict specificity, allows quantitative assessment of the degree of sensitization, and provides a result 3-4 hours after blood sampling [19,20,21].

Procedure for Leukocyte Lysis Reaction (LLR). LLR is performed in test tubes made of chemically pure glass. The antigen used is a suspension of heat-killed brucella (a vaccine strain B. abortus 19BA may be used) at a concentration of 1x10^7 µl/ml. Blood for testing is collected in a volume of 1 ml and placed in a tube with heparin at a rate of 75-80 IU of heparin per 1 ml of blood. The specific leukocyte lysis index (SLLI) is calculated by determining the difference - the percentage of leukocyte reduction in the experimental tube minus the percentage of leukocyte reduction in the control. SLLI is expressed as a negative value and ranges from -10% to -30%. An SLLI less than -10% indicates nonspecific lysis [1,2,3].

It is advisable to highlight the laboratory test complexes used for diagnosing brucellosis based on the goals and level of medical care organization:

1. For epidemiological screening of populations in outbreak areas, the following are used: agglutination reaction (Heddleston reaction), Wright reaction, PHAR, ELISA,

and the Burnet skin allergy test.
2. For population screening before preventive vaccination: the Heddleston reaction or ELISA, the Burnet skin allergy test, or the leukocyte lysis reaction.
3. For diagnosing acute and subacute brucellosis: bacteriological studies, agglutination reaction, PHAR, and ELISA are performed. In cases of negative results, the Coombs reaction is used.
4. For diagnosing chronic brucellosis and during dispensary observation of individuals who have recovered from brucellosis: the Coombs reaction, ELISA, PHAR, and skin allergy tests are recommended.
Serological reactions and the skin allergy test have different diagnostic significance at various stages of the disease and cannot replace each other. This necessitates the use of a comprehensive sero-allergic method, which is the most reliable way to diagnose brucellosis. In the early stages of the disease (within the first 6 months), the diagnostic value of serological methods is higher than that of allergic methods; serological reactions during this period are positive in almost 98% of cases. As the duration of the disease increases, the percentage of positive serological reactions (agglutination reaction, PHAR) begins to decline. In the later stages of the disease, the Coombs reaction, ELISA, and the intradermal allergy test have greater diagnostic value [4,5,6].

It is important to consider that while high antibody titers almost always indicate the presence of infection, low antibody titers or their complete absence do not exclude the possibility of disease. Therefore, repeated testing at intervals of 1-2 weeks is recommended, especially when acute brucellosis is suspected. It should be noted that a positive agglutination reaction with brucellosis antigen may also be given by sera containing antibodies to microorganisms that have common antigenic determinants with brucella [3,4,5,6].

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