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MICROBIOLOGICAL AND VIROLOGICAL TESTING METHODS - ON THE EXAMPLE OF THE CAUSANT OF ANTHROATIA

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Abstract. This article fully describes the methods of microbiological and virological examination - using the example of the causative agent of anthrax. Methods for testing anthrax for allergic skin reactions, hemolytic activity, motility, lecithinase activity, penicillin sensitivity, and obtaining material for testing are described in detail.

Keywords. Luminescent, anthrax, Loeffler's medium, chloroform, hemolytic, phosphatase, lecithinase, proteinase activity, hyperemia.

Introduction. Immunofluorescence method - A luminescent serological method used to observe spores and vegetative cells. A smear is prepared from the suspension of the sample to be examined and fixed on a slide (composition of the fixing liquid: 90 ml of 96% ethyl alcohol and 10 ml of 33% perhydrol). After the smear dries, it is stained with luminescent spore anthrax serum. The slides are placed in a humid chamber (wet filter paper is placed in a Petri dish) and a drop of luminescent serum is placed on top, stained for 15 minutes, washed in 0.9% NaCl solution for 10 minutes, and air-dried. Smears prepared from primary (native) material are stained using the contrast method, that is, a mixture of luminescent serum and bovine albumin labeled with rhodamine is added to the smear. This mixture is prepared in two times the amount. The remaining work is done in the order of staining with fluorescent serum. Before viewing under a microscope, a drop of a liquid consisting of one part 0.15 M (molar solution), NaCl (pH – 7.4), and nine parts glycerol is added to the preparation and viewed under a microscope**.** When viewed with an immersion objective, immersion oil is used and viewed under a microscope at 6 x 90, 7 x 90, 10 x 90. Spores and capsules stained with luminescent serum emit light, characteristic of bacterial morphology. A positive result is considered to be a condition where 2-5 cells are visible under the microscope, with +++ and ++++ fluorescence. Antigen and antibody detection is performed based on erythrocyte diagnostics, using a special guide.

Main part. Polymerase chain reaction - Polymerase chain reaction is performed according to specific instructions provided by the reagent manufacturers.

An allergic skin reaction to anthrax. This reaction is based on observing the allergic reaction that occurs when a patient or someone who has had the disease is injected into the skin with a specific anthrax. The reaction may appear in the first days of the disease, and in some cases on the 7th-8th day of the disease. Anthrax is injected subcutaneously in a volume of 0.1 ml into the forearm, following aseptic techniques. An isotonic solution of sterilized NaCl is injected into the forearm of the other hand in the same amount. After 24 hours, redness and infiltrate are observed at the injection site. If skin hyperemia of 8 mm in diameter is observed at the site of anthrax injection, the reaction is considered suspicious. If the infiltrate with hyperemia is up to 15 mm, then it is weakly positive, if hyperemia is more than 16 mm, and the infiltrate is 8 mm, then it is positive, if the anthrax reaction gives a negative or suspicious result, it is repeated after 5-7 days, this reaction is used for retrospective diagnosis.

Identification of the anthrax pathogen - Identification is performed through the following basic tests: Observation of microbial morphology in preparations prepared and stained on a slide; Special (specific) radiation when stained with fluorescent serum; Capsule formation in primary

pathological material and in the organs of laboratory animals; Penicillin sensitivity ("pearl staining" test); Lysis of the anthrax pathogen with bacteriophage; Pathogenicity in laboratory animals. Capsule formation can be studied in vitro. For this, the culture is grown in a serum-containing medium in the presence of carbon dioxide (Loeffler's medium). Capsule formation begins after 30–120 minutes and is complete after 18 hours of incubation. A paste is prepared from the grown culture. Stained with Romanovsky-Giemza or other methods.

Sensitivity to penicillin. A broth containing 20% inactivated horse serum and 0.5 and 0.5 U of penicillin per ml is added to Hottinger's medium (pH 7.2–7.4) under sterile conditions. The nutrient medium is poured into test tubes in 2-3 ml increments, two drops of a one-day broth culture or a culture grown on a solid nutrient medium are added to a bacteriological Petri dish and inoculated into two test tubes. In the third test tube, only 2–3 ml of sterile broth is placed (for control). The test tubes are incubated for 3–6 hours at 37 0 C, then smears are prepared on glass slides, fixed in Cornui solution (Composition of Cornui solution: six parts ethyl alcohol + three parts chloroform + frozen acetic acid), stained with methylene blue for 20–30 minutes, and viewed under a microscope. In the smear, the anthrax pathogen appears in the form of a round chain resembling a necklace. Bacillus Cereus and other saprophytic bacilli appear normal, while the control forms a chain of rods.

Research observations. Bacteriophage test. A drop of a 5-6 hour culture is placed in a Petri dish filled with meat agar. The lid is left half-open for 30 minutes to dry, and anthrax bacteriophage with a diameter of 2 mm is added to the center of the drop. The reaction is read after incubation at 37°C for 5–6 hours. The final result will be visible after 12-24 hours. A positive result indicates lysis at the site of bacteriophage instillation. Additional identification tests for the anthrax pathogen: determination of hemolytic properties (activity), determination of phosphatase, lecithinase, proteinase activity, etc.

Hemolytic activity - 5.0 - 10.0% (15%) defibrinated sheep blood is added to GPA cooled to 450°C. The culture is plated on agar in the form of a "plaque" and incubated at 37°C for 18 - 20 hours. The anthrax pathogen does not form a zone of hemolysis, while other saprophytic bacilli grow by forming a colorless zone around the "pills".

Determination of motility - For determination, it is inoculated (pricked) onto 0.4% semi-liquid agar and grown in a thermostat at 37 0 C for 24 hours. Anthrax bacteria are non-motile and grow only in the injected (injected) path, while other saprophytes grow by spreading. The food environment becomes cloudy.

Lecithinase activity is determined in Drozhkina's egg broth or Hottinger's GPA with egg yolk. The composition of the yeast medium: one part egg yolk + two parts sterile 0.85% NaCl. 5 ml is poured into test tubes, a one-day culture is planted, and incubated in a thermostat at a temperature of 37 0 C for 18–24 hours. The anthrax pathogen does not break down egg yolk, while saprophytic bacilli do. Preparation of agar with egg yolk: 5-10 ml of chicken egg yolk is added to 100 ml of melted and cooled Hottinger agar to 45 degrees (egg yolk is previously dissolved in a 0.85% NaCl solution in a ratio of 2:3). After mixing thoroughly, pour into Petri dishes. The anthrax pathogen does not form a white, opaque zone around the inoculated colonies, while a wide, white, opaque zone forms around the colonies of saprophytic bacilli. Although the isolated culture does not form a capsule, other specific morphological and tinctorial properties, sensitivity to anthrax phage, and the formation of a "coral glow" in the presence of penicillin make it included in the Bacillus anthracis group and the results of the tests are given.

Research results. Obtaining material for testing: Taking a wool sample. For bacteriological tests, at least 5 wool samples (preferably dirty wool), each weighing 2 grams, are taken from different locations. If the wool is packed in bales, at least 10 samples are taken from different

areas of each bale, as well as dust collected at the seams of the bale. Samples from one bale are packaged as one.

Sampling of leather and fur products. For testing, 3x3 cm pieces of skin (near the areas where the sample was taken for testing and comparison) are taken from peripheral areas of the skin that are not rotten or moldy. If there are areas of bleeding and infiltrates on the inside of the skin, a sample is taken from those areas.

Sampling of concentrated feed. Samples of concentrated feed (grain, bran, mixed feed) are taken depending on their storage conditions. The sample is taken with a dry sterile sampling clamp. At least 5 samples from each batch are taken from an area of 4 m2, 1 sample must be at least 100 g. Initial samples are taken evenly across the entire area, both from the top and from the deep interior. If the feed is in packages (packaged), a sample is taken in units of quantity as follows: when there are 10 packages in a unit of quantity - from each packaged unit: when there are 11 to 100 packages in a unit - from every 10 packaged units and additionally from every 100 packaged units.

Sampling of solid feed. A sample of solid feed (hay, straw) is taken using scissors and tongs, weighing at least 40 g, from an area of 4 m2, at the rate of 1 sample from each different location (set). The collected green mass is taken as solid food, and the primary sample is taken at 100 g.

Sampling of root crops. It is taken depending on the size, 1-3 pieces per 4 m2 of each room. The top part of them is cut off from the places where the soil is stuck and used for testing.

Taking samples from the silo. Sampling of silage stored in bales is done in the same way as sampling of soil and root crops. The resulting primary samples are thoroughly mixed, an average sample is taken from this batch and sent to the laboratory. The average weight of this sample should not exceed 500 grams.

Soil sampling. Soil samples are taken according to plan from May to October. Samples are taken once a quarter from non-stationary plots. The area to be inspected is divided into squares of 4 meters. Using a rotary soil sampler, 20-30 g of soil samples are taken from each end of the square and from the center. Samples are taken from the upper layers of the soil, from a depth of 15 cm, from areas where anthrax pathogens are suspected. Before sampling from graves where the bodies of cattle are buried, a 2-3 cm layer of soil is removed and taken every 25 cm from a depth of up to 2 meters. Soil taken from the pit but not used for sampling is mixed with dry chlorinated lime. In this case, chlorinated lime containing 25% active chlorine is mixed with soil in a ratio of 1:3. It is mixed with the soil, moistened slightly, and thrown into the dug hole. The sampled areas are disinfected with 5% active chlorine, and the tools are burned in the flame of a blowtorch.

Taking water samples. Water samples from natural and artificial open water bodies are taken from the surface (at a depth of 10-15 cm) and from the bottom using a bathometer or a special container. Each sample should be at least 0.5 liters, and the total volume of the water sample taken should not be less than 1 liter. In addition, samples such as mud and soil are taken from the bottom of the water near the shore and tested, and a report is recorded at the site.

Taking swab samples from external environmental objects

To do this, a sample is taken from an area of 100 cm2 with a gauze swab soaked in sterile water. The swab is placed in a glass container containing 4-5 cm3 of sterile water or 0.9% sodium chloride solution and closed with a sterile rubber stopper.

Conclusion. Anthrax is diagnosed when certain clinical signs, epidemiological history and laboratory results are confirmed: If Bacillus anthracis is detected by bacterioscopic methods or isolated from pathological material, and one of two infected laboratory animals dies and Bacillus anthracis is isolated from its organs; If one of two laboratory animals infected with the primary

material dies and Bacillus anthracis is isolated from its organs; If virulent Bacillus anthracis is isolated from the suspected source or transmission agent; Examination deadlines:

Bacterioscopic – the day the sample was submitted;

Bacteriological – up to 3 days;

Biological – up to 10 days. The external environment, food and animal products were examined in the following cases. When it is necessary to identify the source of the pathogen or the factors of transmission of infection from animals to humans. In the relevant instructions, it is used to determine the presence of anthrax pathogens in individual objects, during construction, land reclamation, hydraulic engineering and other works related to the removal and relocation of soil in areas where cattle cemeteries are located, to determine the presence of anthrax pathogens.

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