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DETECTION OF ANTHRACIS IN HUMANS, ENVIRONMENTAL OBJECTS, AND ANIMAL MATERIALS IN LABORATORY CONDITIONS

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Abstract

Anthrax is an acute infectious disease caused by Bacillus anthracis. This disease occurs mainly among livestock and is transmitted to humans through infected animals, their products, and the external environment. This article extensively covers methods for laboratory diagnosis of anthrax, including the steps of sampling and testing clinical specimens (blood, wound secretions, sputum), environmental objects (soil, water), and animal raw materials (hides, wool, meat products). Laboratory diagnostics uses bacteriological, serological, molecular genetic, and immunological methods. Microscopic examination reveals gram-positive rods, and typical colonies are obtained by growing them on special nutrient media. The genetic material of the pathogen is determined using the polymerase chain reaction (PCR) method.

Keywords

Anthrax, Bacillus anthracis, laboratory diagnostics, bacteriological examination, serological method, PCR, epidemiological control, exudate, microscope, disinfection.

Introduction. Anthrax - the skin form of the disease should be examined through fluids released from vesicles, carbuncles, and ulcers. Before taking a sample from the patient, the surface of the carbuncle and the area around the wound are cleaned and wiped with alcohol. The fluid is taken with a sterile pipette, syringe and sterile swab; When a septic form of the disease is suspected, 1 ml of blood is taken from the patient's vein into a clean sterile test tube; When the pulmonary form of the disease is suspected, samples of the patient's stool, urine, and vomit in the form of sputum, intestinal fluid, and urine are taken in a clean, sterilized jar; Damaged organs, tissue fragments, spleen, and of course blood are taken from the corpse for examination. The processes of laboratory examination of pathological material include: Preparation of samples for the necessary examination methods; Microscopic examination of the initial material; Inoculation into feed media; Infection of laboratory animals; Polymerase chain reaction; Identification of isolated cultures. If the pathological material is tissue taken from a wound, it is ground in a mortar (mortar), or softened and crushed in 0.9% saline solution. Crush (grind) in a mortar until 10-12 ml of the mixture is formed.

Main part. Smears are prepared from the obtained materials and fixed in ethyl alcohol with the addition of 3% hydrogen peroxide. By fixation, spore and vegetative forms of Bacillus Anthacis are inactivated for 30 minutes, without affecting the morphology of the microbe and its ability to fluoresce when stained with fluorescent sera. The fixed smear is stained by the Gram method and with fluorescent anthrax serum. The capsule is stained by the Romanovsky-Giemza, Rebiger methods. In Gram-stained smears, Bacillus Anthacis is a straight, Gram-negative rod-shaped bacterium, arranged in short chains or pairs, with the sides facing each other cut flat, and the free sides in the form of a semicircle. In smears prepared from old pathological material, the rods are slightly larger and have rounded ends, the morphological structure of the bacilli is slightly distorted, that is, they are eroded, sometimes remaining in a "shadow", and only barely stained pieces of the shell remain. Initial pathological samples taken from a person (blood, cerebrospinal fluid, exudate, etc.) are cultured on meat-peptone broth (GPB) and meat-peptone agar (GPA). On dark nutrient media, the anthrax pathogen forms flat, dark gray, gadiform

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colonies (R-shape). The centers of the colonies are dark, and the edges are covered with scaly growths. There are also colonies that are not ghadir - bud, and do not have tumors, and they also need to be identified. When the microscope objective is slightly magnified (10-60 times), the colonies appear to be spicules, formed by long strands of microbes, called "jellyfish heads" or "lion's tails." For identification, it is necessary to take smears from colonies with dark spots and spicules on the edges of the culture media and inoculate them on GPSh, GPA, or blood agar in Petri dishes**.**

When a sample inoculated into agar is placed in a thermostat for a day, if anthrax microbes are present, the liquid will become clear (transparent) and a cotton-like precipitate will form at the bottom of the test tube. When the test tube is shaken, it does not become cloudy, but forms small, cotton-like precipitates. In some cases, the CSF becomes slightly cloudy and diffuse microbial growth is observed. When the test tube is shaken, the cotton-like precipitate forms a wavy ripple. Colonies grown on blood agar do not produce hemolysis. Smears are prepared from GPSh, stained by the Gram method, and viewed under a microscope. In smears prepared from cultures in the CSF, chains of anthrax rods are found; when diffuse growth occurs, anthrax rods are found in the smears, either singly or in pairs. When a mixed culture is formed, the anthrax pathogen is isolated using commonly used methods (sowing fragments onto a dark nutrient medium in Petri dishes, plating individual colonies, infecting white mice).

Biological testing (biosampling) is an important method of infecting laboratory animals with primary pathological material, and is performed on the same day as the sample is introduced, along with inoculation into nutrient media. The pathological material to be examined is suspended in a 0.9% NaCl solution and injected subcutaneously (near the tail) into two white mice in an amount of 0.2–0.5 ml. White mice die in 1 - 3 days, sometimes in 10 days. Smears are prepared from the heart blood, spleen, liver, and injection site infiltrate of dissected mice and cultured in nutrient media. To see if a capsule has formed in the smears, they are stained using the Romanovsky-Giemsa method. The culture isolated from the samples inoculated into the nutrient medium is infected with two more white mice in the same manner as described above, one day after inoculation.

Research observations. Packaging and transportation of samples. Each sample is placed in dry, sterile glass jars and sealed with a sterile rubber stopper, cork, or parchment. You can also use polyethylene bags wrapped with twine. The water sample is placed in a sterile jar and sealed with a sterile rubber stopper. The samples are numbered (wool sample - kip number), then packaged in moisture-proof containers and sent to laboratories. The sample order includes the purpose of the inspection, the name and volume of the material, and the location and date of sample collection. In addition to samples taken from wool and feed, their origin, bundle size, type of package, and number of packages are recorded. When sending several samples, the number and place of sampling of each of them is recorded.

From each wool sample, the most contaminated part is taken, cut with scissors and ground, put in a flask with dust, and poured 10 times the amount of 0.9% sodium chloride solution. The flask is closed, shaken well for 10-15 minutes and filtered. Then it is passed through 2-3 layers of gauze to remove the crushed pieces; 1 g pieces of skin from different areas brought to the laboratory are crushed in a porcelain mortar and poured into a volume of 0.9% sodium chloride solution to obtain 10-15 ml of the mixture. After some time (after the material has softened), the pieces are chopped with scissors and kept at room temperature for 2-3 hours. The sample is then thoroughly crushed in this solution until it separates into fibers, then the fibers are pressed against the inner wall of the mortar and removed; A sample of feed (50-100 g) is placed in a flask (crushed with scissors for solid feed) and a solution of 0.9% sodium chloride is added in an amount that produces a wash. The flask is closed and shaken for 10-15 minutes. After standing for 5-8 minutes, it is filtered through 2-3 layers of gauze without any lumps; Soil samples are cleaned of roots and stones, mixed thoroughly. Take 50-70 g of each sample, put it in a flask, add 0.9% sodium chloride solution and distilled water to a volume of 15-20 ml, mix thoroughly

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for 25 minutes, let stand for 5-8 minutes, then filter through 3-4 layers of gauze; Water with underwater plants is filtered through 2-3 layers of gauze. Clean water is tested without prior preparation; When washing from external environmental objects, used swabs are pressed against the inner wall of the test tubes, then they are removed and used to examine the remaining liquid in the test tube; The material contaminated with foreign microflora is divided into two parts. The first part is not heated (cold method).

If filter membranes are not available for hot and cold methods, the washes are centrifuged at 3000 rpm for 15 minutes, and the supernatant is removed. The sediment is sown in simple nutrient media. Supplements for Bacillus anthracis are added and white mice are infected.

Conclusion. Bacillus anthracis culture in the following cases: The specific nature of the anthrax pathogen and the death of at least one of the two animals infected with the original material or the culture isolated and the detection of capsular bacilli in a smear prepared from its organs; Death of one of the infected laboratory animals and isolation of the causative culture from its organs, its specificity and susceptibility to anthrax phage and penicillin; A distinctive feature of the isolated culture is its non-virulence to white mice, but its sensitivity to the anthrax phage and penicillin. Also of particular importance are compliance with biosafety rules, disinfection measures, and the protection of laboratory personnel. The article provides recommendations for early detection of anthrax and strengthening epidemiological control, preventing the spread of the disease, and improving sanitary and hygienic measures. The research results have important scientific and practical significance in the activities of healthcare, veterinary, and sanitary-epidemiological systems.

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