DIAGNOSTIC ISOLATION MICROBIAL CULTURE AND METHODS

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Abstract: In this article, detailed information on the methods of planting and replanting microorganisms in a dense and liquid nutrient medium was obtained and studied. First, we put 10 ml of sterile physiological solution in every 2-3 tubes 9 ml of GPA in 5-6 test tubes, graduated pipettes and 5-6 sterile Petri dishes, a mixture of several types of bacteria in a test tube (staphylococci, salmonella, hay bacillus). Cells of microorganisms grown in a liquid nutrient medium are taken in a sterile pipette, less often - with a needle. In order not to damage the cells of microorganisms, the hook (needle) is cooled by touching the inner surface of the container or the place where the microbe does not grow in the nutrient medium, and only then a smaller amount of microbial mass is taken. comparison of the results was studied.

Key words: sterile physiological solution, Petri dishes, pure culture,: Pasteur, Koch, Drigalsky, physical, chemical and biological methods, pathogenic anaerobes, disinfectant solution, Biotest method, proteolytic properties, lead acetate, indicator paper 'oz, sodium bicarbonate, distilled water, MPB, oxalic acid, GP, etc.

In today's modern world, like all fields, biotechnological processes and the processes implemented on them are being fully studied. For example, in laboratory practice, when some materials are examined bacteriologically, there may be a mixture of two or more types of microbes. A microbe of one species isolated from it is called a soft culture. Isolation of a pure (one type) culture of microbes is the main work of bacteriological tests. Only its pure culture is used to study the properties of microbes and to determine their type. In order to isolate the pure culture, bacteria are grown in separate colonies (in a dense nutrient medium) in special seeding methods. Given that a colony is formed by the multiplication and development of a single microbial cell, a pure culture allows isolation from a single colony when re-inoculated into a sterile nutrient medium. There are different methods of separation of pure culture: Pasteur, Koch, Drigalsky, physical, chemical and biological. In the Pasteur method, take 9 ml of GPB in 8-10 test tubes, add one drop from the test sample to the first one with a pipette, mix and add 0.1 ml of it transferred to the second and subsequent test tubes and mixed, diluted to the last test tube. As the degree of dilution increases, the number of microbes decreases.

Pasteur thought that a type of germ would remain in the last test tube. But this method is less likely to isolate a pure culture. Currently, Pasteur's dilution method is

used as an auxiliary method in performing other methods. Koch's method, 10-15 ml of GPA dissolved in 5-6 test tubes and cooled to 45-50°C are tested, and the tested material is diluted in them one by one and placed in separate Petri dishes from each test tube. After the environment freezes, the bowls are turned over and placed in a thermostat for 18-34-48 hours. In the last bowls, the pure culture we are interested in grows in the form of individual colonies. Sterile GPB from an individual colony. GPAs are seeded. Using Koch Pasterusuli, only water, milk, dung, etc., using solid nutrient medium instead of liquid medium. used in the inspection of materials

Drigalsky method Petri dishes with 5-6 GPA are obtained. A drop of test material is placed in the center of the medium in the first cup and applied to the surface of the medium with a glass spatula. The residual material on the spatula is transferred to the second cup and applied to the surface of the medium, etc. to the last calyx. Then the cups are placed in the thermostat. Individual colonies grow in the last cups, and a pure culture is separated from them by selectively replanting them in a sterilized nutrient medium. A bacterial hook can also be used instead of a spatula. In this case, the material is planted in the form of zigzag or dashed lines. The physical method is often used to separate spore forms of bacteria from non-spores. The tested material suspension is heated in a water bath at 80°C for 30-40 minutes. Vegetative bacteria die, spores remain. Testing is continued by Drigalsky or Koch methods. Chemical method - when a certain amount of chemicals are added to the nutrient medium, some types of bacteria die (have a bactericidal effect), some of them stop growing. (bacteriostatic) other species grow well without being affected by chemicals.The use of selective and elective environments is also based on this. The biological method is used to isolate a pure culture of pathogenic microbes: a susceptible laboratory animal (white mouse, guinea pig, rabbit) is infected with a suspension of the tested material (tissue, bacteria). If there is a pathogenic microbe in the material, the animal will get sick and die. When the dead animal is cut open and its internal organs are planted in nutrient media, the soft culture of the pathogenic microbe is separated. Shukevich's method - when the material is planted in a condensate drop of GPA, mobile bacteria grow to the top of the medium and only a few are removed from it. if planted in a clean nutrient medium, a pure culture of a mobile bacterium is isolated. But special anaerobic microbe growing media is used.

Drigalsky's method - instead of GPA in Petri dishes, special blood-glucose GPA is used, anaerobic conditions are created (desiccator, microanaerostat). Wilson-Blair method of sowing - black colonies grow individually in the nutrient medium. Kitt- A soft culture is isolated when replanted in Tarossi medium. Bioassay method - when susceptible laboratory animals are infected with the tested material or mixed culture, they become sick and die. Pathalogoanatomical incisions are made, their internal organs are planted in Kiti-Tarossi medium, semi-liquid agar or blood-glucose agar, and

A pure culture of pathogenic anaerobes is isolated using one of the indicated pure culture isolation methods. Pure culture isolation method (Drigalsky method)

1. Experience. The first day: the material tested by the Drigalsky method is planted in a solid medium placed in 3 Petri dishes. For this, a glass spatula is wrapped in paper and prepared in a sterilized manner: 1. Drop a drop of the material under test on the surface of the first marked nutrient medium. it is dripped with the help of the inside and applied with a sterile spatula, first backwards, then forwards. After that, the nutrient medium is distributed over the entire area with a circular motion. When doing this, the lid of the bowl opens up to a height where the spatula will fit.

The spatula is removed from the first marked cup, then the lid is quickly closed and the spatula is immediately placed in the second marked cup. In this case, it will not burn. The material is distributed with a spatula, as it was distributed in the bowl with the first mark, and the second bowl is also closed. After that, the spathe is placed in the bowl with the third mark and with this side the material is spread over the surface of the nutrient medium, the bowl cover is closed. After that, the spatula is burned or placed in a disinfectant solution, the patient's last name and date are written, and it is placed in the thermostat with the base up and the cap down. Cups are placed in a thermostat for 18-24 hours. Purpose of the work: Methods of sitting and replanting microorganisms in a dense and liquid nutrient medium. Materials and equipment: gas stove, alcohol lamp, Pasteur pipette, special brush, flask, Petri dish, milk, GPB, carbolic acid, lysol, formalin. Milk, butter, straw. Silage, water, pus, and dead animal tissues are planted in sterile nutrient media to obtain bacterial cultures. Of course, this process is performed near the flame of a burning gas or alcohol lamp in order to prevent bacteria from entering during planting. Planting is carried out with a hook or Pasteur pipettes. Each material brought to the laboratory for examination is recorded in special logs. Before sowing, the examination number, the name of the microorganism and the sowing date are written on the test tube (tube or Petri dish) with a special brush.

Workflow: Microbial inoculation techniques. If microorganisms are grown in a dense nutrient medium, bacteriological material for cultivation or drug preparation is taken with a bacteriological hook or needle; sterile pipettes are used to remove cells from the liquid nutrient medium. The bacteriological loop is made of a thin platinum wire, which is attached to a metal handle. The diameter of the bacteriological loop is 1.5-3 mm. Bacteriological loop (needle) is sterilized before collecting microorganism cells. To do this, the wire is heated until it becomes red in the flame of a burning gas or alcohol lamp up to the point of connection with the holder. In this case, for uniform sterilization, the loop is held vertically in the flame, and then it is quickly placed in a container with microorganisms. In order not to damage the cells of microorganisms, the loop (needle) is cooled by touching the inner surface of the container or the place where the microbe does not grow in the nutrient medium, and only then a small amount

of the microbial mass is collected in the test tube. cells of microorganisms grown in a dense nutrient medium are obtained as follows: the culture tube is held in the left hand, the surface of the nutrient medium on which the microorganisms grew should be facing up and well visible. The test tube is held horizontally or slightly inclined. The hook is held in the right hand like a pencil and is heated in the flame of a torch. Then, without letting go of the hook, the outer side of the cotton-gauze plug is pressed against the palm of the hand with small and ringless fingers, and the plug is removed from the test tube. The edges of the open tube are lightly heated over a flame, a sterile loop is inserted into the tube, less micromassage is performed, and the loop is removed from the tube. The edges of the test tube are heated again in the flame of the burner, then the side of the cotton-gauze stopper that goes inside is also passed through the flame, and the test tube is closed and placed on the stand. The obtained material is used for the preparation of the drug. Cells of microorganisms remaining in the loop are burned in the flame of the heating pad. Cells of microorganisms grown in a liquid nutrient medium are taken in a sterile pipette, less often - with a needle. A sterile pipette is taken with the middle and thumb fingers of the right hand, a test tube (flask) with a liquid medium is held in the left hand, and the pipette is injected into the liquid, taking the above precautionsTake a portion of the medium and close the test tube with a stopper. The obtained sample is used to prepare a drug or to plant in a new nutrient medium. Then, without touching the surrounding objects, the used pipette is quickly placed in a disinfectant solution (0.5-3% solution of chloramine in water or 3-5% solution of phenol in water). For seeding and replanting cells of microorganisms from one environment to another, two test tubes are placed in the left hand - one with nutrient medium (far from you), the other with a culture of microorganisms (close to you), and a bacteriological loop is taken in the right hand. The hook is sterilized in the flame of the burner, then the test tubes are opened by pressing the stoppers of both test tubes with the little and ring fingers of the right hand. Cells of microorganisms are suspended with a bacteriological hook and the hook is brought to the inclined sterile nutrient medium almost to the bottom of the test tube; the loop is moved upwards with zigzag or straight (dash) linear movements. Sowing with needles is carried out in the same way, only the needle is inserted vertically into the nutrient medium. For planting in liquid media, the test tubes are held almost vertically so that the stoppers do not come into contact with the liquid and do not get wet. The loop is placed in the correct nutrient medium with microorganism cells. All the above-mentioned processes are carried out as quickly as possible near the flame of the burner (not in the flame!). This prevents foreign microorganisms from entering the culture. It is impossible to move quickly, to walk next to the person planting the microorganisms, because air movement can cause contamination of the culture. When planting in liquid nutrient media (milk, GPB), the test tube is held in the left hand as when preparing smear preparations; a hook (or

pipette) with the material to be planted in the right hand and corks of test tubes are taken. Next to the burner flame, a hook (or pipette) with a drop of material is gently dipped into the sterile medium in the test tube. The test tube is closed with a stopper, the loop is burned in the fire, and the Pasteur pipette is placed in a disinfectant solution (carbolic acid, lysol, formalin). During operation, the liquid should not touch the stopper of the test tube and should not spill. The ready-planted nutrients are placed in a thermostat. Culture and sterile nutrient medium (GPA) tubes that are replanted when inoculated into dense medium are held obliquely in the left hand (slanted surface of the agar above) with the stopper facing the burner flame. A loop is carefully inserted into a test tube of culture or other material opened near the flame of the burner, and a small amount (one drop) is taken by lightly touching the tested material, and transferred to a test tube with a sterile nutrient medium. The hook is brought to the bottom of the test tube, dipped in the condensate liquid and rubbed with zigzag movements up to the surface of the slanted agar.When planting vertically in a dense environment, the test tube is held horizontally. Seedlings (test tubes) are grown in a thermostat. After 16-18, 24-48 hours, the result is taken into account and the cultural characteristics of the bacteria are studied. The growth of microorganisms in a liquid nutrient medium is manifested by uniform turbidity and sedimentation (in this case, the medium becomes clear) due to the multiplication of bacterial cells. Some types of microorganisms have a high demand for air oxygen, and they grow by forming a film on the surface of the liquid medium, in which the broth does not become cloudy. In a number of cases, bacterial cultures simultaneously turbid the medium, form a large sediment, and form a ring on the wall of the test tube. In dense nutrient environments, cultural characteristics are determined depending on the character of the growing colony. If a large number of bacterial cells are planted on the surface of the environment, the microbe will spread and grow. If fewer cells are planted on a large surface of the nutrient medium, each bacterial cell divides and reproduces to form a separate colony. Depending on the diameter of the colon, they are large or small. it can be dewy. Colonies of most types of bacteria, actinomycetes, and molds can be colored in different colors when they grow in different nutrient media. This is expressed by their production of pigments. If the pigment is soluble, the medium is completely colored, if it is insoluble, only color, blue-green, white, yellow, red, etc. It is characteristic that it forms a pigment. Pigment formation is best shown in dense nutrient environments. Temperature is also important in this; 25-30°C is optimal for most species. Air oxygen and light rays are also affected to a certain extent. Studying the biochemical properties of microbes (oxidation, fermentation, fermentation) in a diagnostic laboratory. Some microorganisms secrete proteases when growing in a nutrient medium, their effect under it, protein molecules are divided into intermediate decomposition products - peptones, albumoses, polypeptides. Under the influence of other enzymes, these

products are divided into separate amino acids. Some pathogenic types of microbes with specific proteolytic activity break down proteins to final products - indole, hydrogen sulfide, ammonia. Indole and hydrogen sulfide are of the greatest importance in determining the type of microorganism. To study the proteolytic properties of microbes, the studied culture is inoculated into a nutrient medium containing one or another protein, for example, MPB, MPZH, milk, brain medium, etc. To study the proteolytic properties of microbes, the studied culture is inoculated into a nutrient medium containing one or another protein, for example, MPB, MPZH, milk, brain medium, etc. Reagents and tools: Lead acetate, indicator paper, sodium bicarbonate, distilled water, MPB, oxalic acid, GPJ. Procedure for performing laboratory work. Definition of hydrogen sulfide. A strip of indicator paper impregnated with lead acetate (lead acetate) is placed under the stopper of the tube with MPB and the culture being studied. The indicator paper should not touch the cultural environment. In positive cases, the hydrogen sulphide formed combines with colorless lead acetate. Lead sulfide is formed and gives the indicator paper a dark brown color (Indicator paper recipe: filter paper strips are placed in a solution with the following composition - distilled water 100 ml, lead acetate 20 g, sodium bicarbonate 1 g. the paper is dried, cut into strips 4 cm wide and 5-6 cm long. Store in a dark glass container). Determination of hydrogen sulfide. Kligler, Olkenitskyi can also be done considering the growth of culture in the combined dense environment. If hydrogen sulfide is released, then in these environments it interacts with iron sulfate (Mohr's salt). Black iron sulfide is formed, the column of the medium turns black. Definition of indole. Indole can be determined in different ways. The most convenient and convenient method is using indicator papers prepared according to one of the following recipes: the filter paper is impregnated with a 12% hot water solution of oxalic acid, air-dried, cut into strips and stored in a dark glass container. To determine indole, MPB o A sheet of paper is placed under the stopper of the test tube. In the presence of indole, the lower part of the paper is colored light pink; filter paper is impregnated with a warm solution consisting of paradimethylaminobenzaldehyde - 0.5 g, ethyl alcohol 96% - 50 ml, concentrated phosphorous or hydrochloric acid - 10 ml (Ehrlich's reagent). The paper is dried, cut into strips and stored in a dark glass container. Paper color is yellow. In the presence of indole, the lower part of the paper turns from gray-pink to dark red. Indole can be detected using Ehrlich's reagent without using indicator papers. For this purpose, an equal amount of ether is added to 1 ml of 2-day-old broth culture and shaken vigorously. Then Ehrlich's reagent is poured into the test tube dropwise along the wall. In the presence of indole, a bright red ring forms at the interface between the ether and broth cultures. Biochemical properties. Studying the biochemical properties of bacteria is an important differential diagnostic method for identifying infectious disease agents. The saccharolytic properties of bacteria are determined by planting them in a

differential-diagnostic nutrient medium containing various carbohydrates and indicators. For this, the culture is grown in Gissa nutrient medium (containing -glucose, lactose, maltose, sucrose, mannitol, dulcite, arabinose, sorbitol, etc.) in sterile skimmed milk, litmus milk, milk with methylene blue added. In the thermostat the result of fermentation of carbohydrates is taken into account. For this purpose, semi-diluted agar with added carbohydrates and indicators, as well as Endo, Levin, Ploskirev dense nutrient mediums are used. Proteolytic properties are often studied by growing the culture in GPJ. Gelatin undergoes proteolysis under the influence of bacterial enzymes and dissolution (liquefaction) occurs in the medium. Different types of microbes dissolve gelatin differently. Some are funnel-shaped, some are bag-like, sock-like, etc. Bacteria are determined by the formation of end products of protein degradation (indole, hydrogen sulfide, ammonia, etc.). Specially prepared litmus papers are used for this. Paper dipped in lead acetate solution darkens under the influence of hydrogen sulfide, pink litmus paper turns blue under the influence of ammonia, and yellow indicator paper turns pink under the influence of indole. Second day: In order to study the accumulation of microbes and to separate the pure culture, after 18-24 hours, the cups are removed from the thermostat and the cultured material is studied. Because there is a lot of material in the first cup, the bacteria grow evenly. A separate microbe collections are not visible. In the second calyx, and especially in the third calyx, germ bundles are located separately. Therefore, it will be convenient to study them. 1. Microbial collections are studied macroscopically. The cup is inspected without opening using the light passing from the side. Size of the collection: large, small, point size; shape: straight, round, curved, flat convexity is studied. Consistency: hard, soft; We will study its smooth, smooth, shiny, dull, moist, sandy, mucous surface. 2. Magnifying the collection of microbes with the help of a microscope eyepiece or a magnifying glass makes it possible to study it better. To better study the microbe, it is necessary to narrow the aperture of the microscope or lower the condenser. 3. A smear is prepared from the selected collection of microbes and then stained by the Gram method. Some marked collections are planted in slanted, agar tubes and placed in a thermostat for 18-20 hours. The number of microbe collections is determined. Third day: purity of the culture and characteristics of microbes are studied. After a few days, the test tubes are removed from the thermostat to ensure that a type of microbial culture has grown. All the results were obtained, compared, studied and the results were compared.

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