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Physiology of microbiology

Practicum on Microbiology, Virology and Immunology for English-speaking students II years of the medical faculty, specialty "Medicine"

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МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ

Запорізький державний медичний університет

Кафедра мікробіології, вірусології та імунології

Фізіологія мікроорганізмів

Практикум з мікробіології, вірусології та імунології

для англомовних студентів
II курсу медичного факультету,
спеціальність «Медицина»

Запоріжжя - 2019

Практикум з мікробіології, вірусології та імунології для англомовних студентів II курсу міжнародного факультету, спеціальність «Медицина».

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STERILIZATION AND DISINFECTION

Theme topicality. In practice it is often necessary to control undesirable growth of microorganisms, to limit their signs and speed, either partially or completely neutralize bacteria in the external environment or tissues. There are a lot of ways to do this at the present stage of medicine development. Knowledge of basic methods of sterilization, disinfection, and antisepsis is the earnest of success in the work of a doctor

.

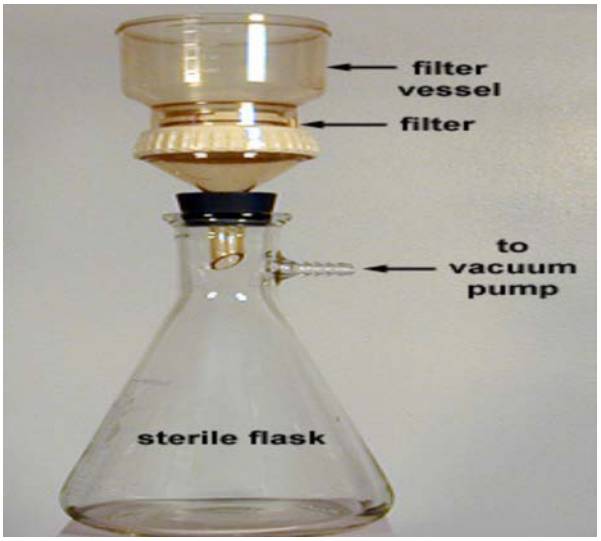
Primary objective: to be able to use basic knowlage of microbiological disinfection, sterilization, and antiseptics in medical practice.

QUESTIONS FOR DISCUSSION

1. Sensitivity of microorganisms to physical and chemical factors.
2. Effects of physical factors on microorganisms: filtration, drying, radiation, ultrasound, and temperature.
3. Effects of chemical factors on microorganisms: phenols, halogens, alcohols, acids, alkalis, oxidizers, aldehydes.
4. Disinfection: definition, purpose, types (physical and chemical methods). Disinfection effectiveness monitoring.
5. Microbiological basis of aseptic and antiseptics.
6. Sterilization: definition, purpose, types (thermal methods and monitoring of the impact of ionizing radiation, filtering, and chemical methods). Control of sterility of medical equipment.

PROCEDURE OF PRACTICAL WORK

Task 1. Study the demonstration.



Seitz and Berkefeld filters

Seitz and Berkefeld filters are bacterial filters used for sterilization of liquids by filtering in the case where the material can not be warmed.

Filters are made of finely porous substances and asbestos. Filtering is conducted at high pressure or vacuum creates conditions for acceptance.

For sterilization of liquids, they use filters with pores, diameter of which is smaller than viruses.

This method is used in the biotechnological production in the manufacture of vaccines, immune sera, solutions of antibiotics, bacteriophages, enzyme solutions, vitamin solutions, etc.

Рис. 25. Автоклав ГК-75

Autoclave

Autoclave. Moist heat sterilization must be carried out at temperatures above 100 °C in order to destroy bacterial endospores, and this requires the use of saturated steam under pressure. Steam sterilization is carried out with an autoclave**,** a device somewhat like a fancy pressure cooker.

The development of the autoclave by Chamberland in 1884 tremendously stimulated the growth of microbiology. Water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber.

The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed.

Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually 121 °C and 15 pounds of pressure.

At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes. Treatment is continued for about 15 minutes to provide a margin of safety. Of course, larger containers of liquid such as flasks and carboys will require much longer treatment times.

Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins. It also may disrupt cell membranes. Autoclaving must be carried out properly or the processed materials will not be sterile. If all air has not been flushed out of the chamber, it will not reach 121°C even though it may reach a pressure of 15 pounds.

The chamber should not be packed too tightly because the steam needs to circulate freely and contact everything in the autoclave. Bacterial endospores will be killed only if they are kept at 121 °C for 10 to 12 minutes.

When a large volume of liquid must be sterilized, an extended sterilization time will be needed because it will take longer for the centre of the liquid to reach 121°C; 5 liters of liquid may require about 70 minutes. In view of these potential difficulties, a biological indicator is often autoclaved along with other material.

This indicator commonly consists of a culture tube containing a sterile ampule of medium and a paper strip covered with spores of *Bacillus stearothermophilus* or *Clostridium* PA3679. After autoclaving, the ampule is aseptically broken and the culture incubated for several days. If the test bacterium does not grow in the medium, the sterilization run has been successful. Sometimes either special tape that spells out the word *sterile* or a paper indicator strip that changes colour upon sufficient heating is autoclaved with a load of material. If the word appears on the tape or if the colour changes after autoclaving, the material is supposed to be sterile.

Sterilizator. Koch's apparatus.

Sterilizator. Koch's apparatus. Sterilizator is used to sterilize by means of boiling surgical instruments, syringes, needles, etc.

Koch's apparatus is used for fluid steam sterilization. Nutrient medium is sterilized in it with the addition of native proteins.

Discontinuous sterilization lasts for three days for 30 minutes at temperature 100 °C.

In between sterilizations of nutrient medium is left at room temperature. During the first heatingthere is a loss of vegetative bacteria forms, spores remain viable.

On the next day spores germinate in vegetative form at room temperature, which were killed at repeated heating.

Pasteur stove. Pasteur stove is used for by dry-heat sterilization of glassware. Glass is prewraped in paper. Petri dishes and pipettes can be sterilized in the metal case.

Temperature rate of sterilization:

+ 150 °C – 2 h.
+ 160 °C – 1 h.
+ 180 °C – 30 min.

Task 2. Determine antibacterial action of disinfectants.

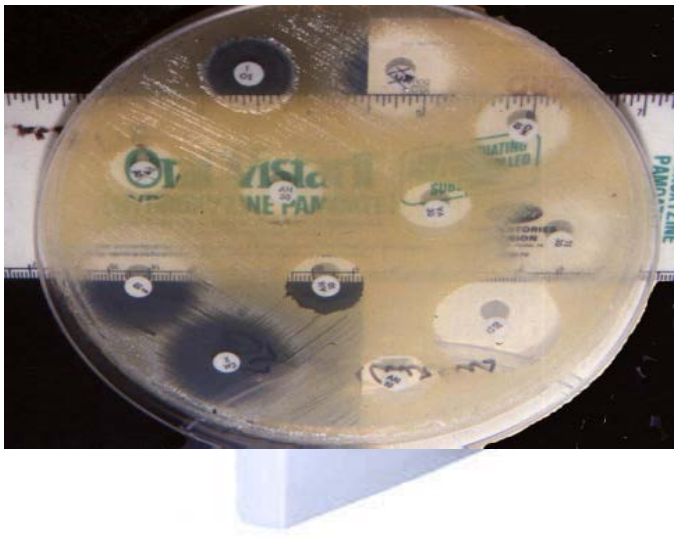


Рис. 26. Сухожарова шафа

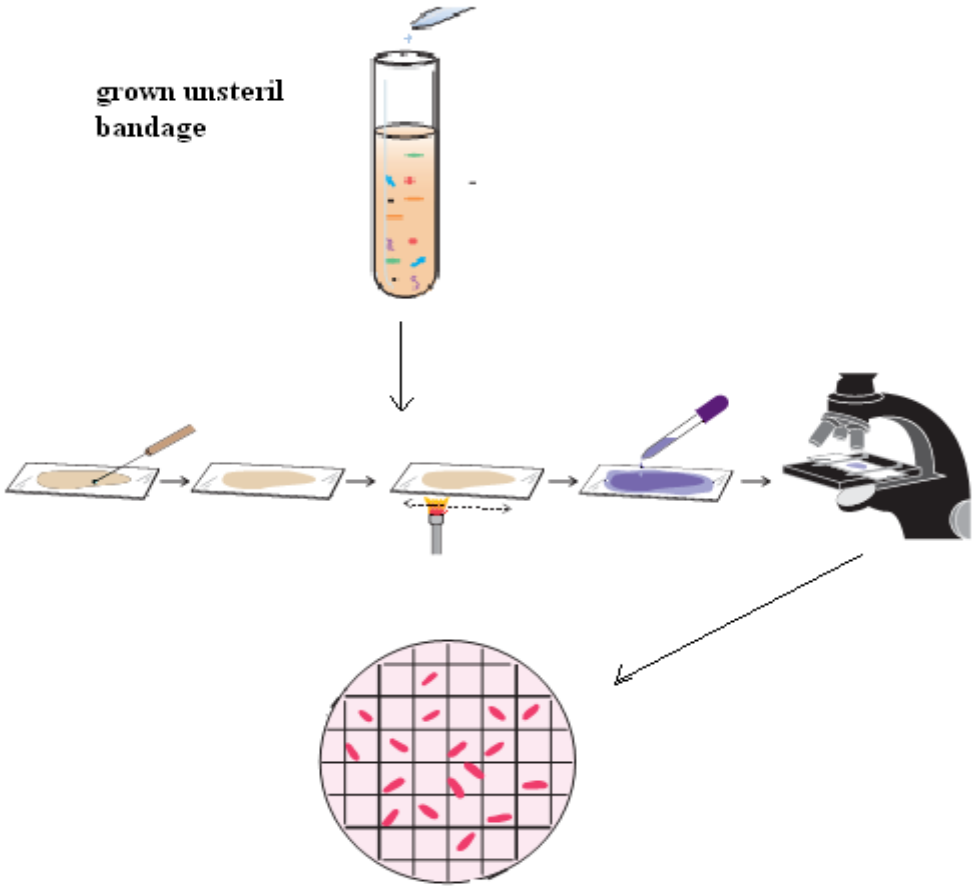
Antibacterial action of disinfectants

For this experiment, on a nutrient medium there was grown a pure bacterial culture. Small filter paper discs containing the desinfectant to be tested are then placed on the surface of the medium.

After incubation, a film of growth will cover the plate, but a clear zone will surround those discs that contain an inhibitory compound. The size of the zone reflects several factors, one of which is the effectiveness of the inhibitory agent. Explore this area and make a conclusion about the effectiveness of disinfecting solution with respect to this pure bacterial culture.

Task 3. Study bacterial contamination of unsterile bandage.

Procedure of study: one thread from the unsterile bandage was put into sterile peptone water. A test tube was cultivated in a thermostat at 37 °C for one day.



Procedure of unsterile bandage investigation

Table. Sterilization and Disinfection

Notion	Definition/explanation
Antimicrobial agents	1. Physical agents: temperature (high or low); radiation, filtration, desiccation, osmotic pressure. 2. Chemical agents: disinfectants, antiseptics, sanitizers, antibiotics. 3. Biological: antibiotics, bacteriophages
Microbe consideration	1. Endospores are extremely heat-resistant. 2. Gram-negative microbes are more resistant to disinfectants and antiseptics than gram-positive ones

Table 1.4.1 continuation

Notion	Definition/explanation
	3. Bacteria in stationary phase in general are more resistant than in log phase. 4. Mycobacteria, protozoan cysts, and oocysts are very resistant to disinfectants and antiseptics than enveloped viruses. 5. Non-enveloped viruses are generally more resistant to disinfectants and antiseptics. 6. Pseudomonas can metabolize many chemicals
Reduction in numbers	1. Disinfectant is used to disinfect inanimate objects. It is too toxic to be used on human tissues. 2. Antiseptic is an agent that kills or inhibits growth of microbes. It is safe to be used on human tissue. 3. Sanitization is cleaning of pathogenic microorganisms from public eating utensils and objects. 4. Decontamination is treatment of an object or inanimate surface to make it safe to handle
Sterilization	Sterilization is the complete destruction or removal of all living organisms from the object being sterilised. The development of methods of sterilization was mainly a consequence of the controversy over spontaneous generation

	culminating in the work of Pasteur. Experiments designed to prove or to disprove spontaneous generation depended upon two general principles: 1) the complete sterilisation of a suitable growth medium so that no living organisms exist at the start of the experiment, 2) the design of the vessel of a type that it is impossible for microbes to enter from outside
Method of sterilization	1. Physical. 2. Mechanical (filtration). 3. Chemical
Dry heat sterilization	1. Direct flame (incineration). 2. Hot air oven: a) radiating dry heat is used for sterilization; b) 160 ^{o}C for 2 h.; c) advantage; d) proteins oxidiation and cells dehydration
Moist-heat sterilization	1. General principles: a) penetration of materials by moist heat is generally much more rapid than by dry heat because water conducts heat better than air; b) moist heat kills microorganisms by denaturing their proteins. 2. Boiling water: a) sterilizing agent is not considered b) the absolute minimum exposure period should be 30 minutes. 3. Autoclave: a) moist heat in the form of pressurized steam; b) typical operating condition for sterilization is 15 psi (pounds per square inch) at 121.5 $^{\circ}$C for 15 minutes; c) limitations: the majority of plastics melt in the high heat; sharp instruments often become dull; oily substances cannot be treated since they do not mix with water; many substances break down during the sterilization process; d) testing sterilization effectivness: film-containing spores of *Bacillus stearothermophilus* or *Bacillus subtilis* 4. Pasteurization: a) mild heating that is sufficient to kill particular spoilage or

	disease organisms without serious damage of the of the product taste; b) pasteurization is not sterilization;

Table 1.4.1 continuation

Notion	Definition/explanation
	c) primarily aimed at destroying *Coxiella burnettii* (cause of Q fever); d) holding method or (LTLT), low temperature for a long time is 62.9 °C for 30 minutes; e) high temperature for a short time (HTST) or flash pasteurization is 71.6°C for 15 seconds; f) ultrapasteurization is 82°C for 3 seconds; g) ultra-high-temperature (UHT) is steam at 140 °C for 3 seconds (sterilization); h) also used to eliminate *Salmonella* and *Escherichia coli* that can contaminate fruit juices; i) egg pasteurization. 5. Canning: a) blanching steam heat for 3–5 minutes; b) canning all air is removed and can is placed in a pressured steam sterilizer at 121 °C for a specified amount of time, depending on the product
Materials that can be autoclaved	Decontamination of cultures, cells, glassware, pipettes. Sterilization of media, labware, dressings, surgical instruments, polycarbonates, polyethylene
Materials that can't be autoclaved	Heat sensitive solutions: sugars, salt, antibiotics, drugs. Plastics
Filter sterilization	Depth filters : Layer upon layer of fibrous materials that form a band. You can do it with relatively high volume, with a high flow and a relatively high level of turbidity. HEPA filters are important for a number of biomedical applications.

	Air from labs that might contain pathogens is HEPA-filtered and then released into the atmosphere. Animals in labs are sometimes housed in HEPA-filtered environments. Membrane filters: These serve as a surface against which you can trap particles. Some of these filters are very large and of high capacity. Small-scale membrane filters are often used in labs, such as for solutions or buffers. Rotating membrane ultrafiltration (RMU). Vortex-flow filtration (VFF)
Chemical sterilization	Chemicals have also a place in sterilization. Heating provides the most effective ways to rid and object of all transmissible agents, though it is not always appropriate, because of the material an object is made of. (Such as plastics, fiber optics, and electronics)
The effect of light and radiation	Some bacteria withstand the effect of light fairly well, while others are injured. Direct sunlight has the greatest bactericidal action. Investigations have established that different kinds of light have a bactericidal or sterilizing effect. These include ultraviolet rays (electromagnetic waves with a wave length of 200–300 nm), X-rays (electromagnetic rays with a wave length of 0.005–2.0 nm), gamma-rays (short wave X-rays), beta- particles or cathode rays (high speed electrons), alpha-particles (high speed helium nuclei) and neutrons. Viruses are very quickly inactivated under the effect of ultraviolet rays with a wave length of 260–300 nm. These waves are absorbed by the nucleic acid of viruses and bacteria. In result of action UVR DNA are damaged. Viruses in comparison to bacteria are less resistant to X-rays, and gamma-

Table 1.4.1 continuation

Notion	**Definition/explanation**
	rays. Beta-rays are more markedly viricidal. Alpha-, beta-, and

	gamma-rays in small doses enhance multiplication but in large doses they are lethal to microbes. Ionizing radiation can be used for practical purposes in sterilizing food products, and this method of cold sterilization has a number of advantages. The quality of the product is not changed as during heat sterilization which causes denaturation of its component parts (proteins, polysaccharides, vitamins). Radiation sterilization can be applied in the practice of treating biological preparations (vaccines, sera, phages, etc.)
Preservation of perishable products	Preventing or slowing the growth of microbes extends the shelf life and can be achieved using various methods: 1. Use of preservative chemicals: - non-food items use germicides (i.e., formaldehyde in shampoo); - food items use weak acids (i.e., benzoic, sorbic and propionic acids.); - nitrates/nitrites are typically used to preserve meat (inhibit the germination of endospores and growth of *Clostridium botulinum*). 2. Low-temperature: - slows enzymatic reactions; - freezing forms ice crystals that damage microbial cells. 1. Reducing water availability: - salt or sugar addition: high solute environment causes plasmolysis and prevent growth of microbes). However, *Staphylococcus aureus* (food poisoning organism) can grow under very high salt concentrations; - food drying (dessication, lyophilization): however, drying does not kill microbes. Number of salmonellosis cases are sometimes reported when dried eggs or their products are used -
Microbial form which is the	If you have been even half awake for the microbiology

most resistant to sterilization	lectures so far, you should have a clear picture in your mind that microorganisms (germs if you insist) are extremely variable in structure. Bacteria are single cells, virusus are simply pieces of DNA in a coat of protein, and most fungi are multicellular with a strong cell wall. With microorganisms being so variable in structure and composition, it is predictable that they will not all respond the same way to physical and chemical methods designed to kill them (disinfection and sterilisation). Some bacteria can produce a long term dormant survival stage called a spore. This structure is the most resistant microbial form to the action of heat and chemical disinfection and sterilisation processes.
Disinfection	Disinfection and the use of chemical disinfectants is one key strategy of infection control. Disinfection refers to the reduction in the number of living microorganisms to a level that is considered to be safe for the particular environment. Typically, this entails the destruction of those microbes that are capable of causing disease. Disinfection is different from sterilization, which is the complete destruction of all microbial life on the surface or in the liquid. The steam-heat technique of autoclaving is an example of sterilization
Disinfectants	Not safe for application to living tissues. Examples: chlorine, hypochlorites, quaternary ammonium compounds
The ideal disinfectant	1) broadly active; 2) resistant to inactivation; 3) not poisonous or otherwise harmful; 4) easy to prepare and stable;

Table 1.4.1 continuation

Notion	Definition/explanation
	5) penetrating; 6) cheap and easy to obtain and use; 7) appropriate for the conditions
Chemical disinfectants	1. Alcohols: – aqueous solutions of 60–80% ethanol/isopropanol rapidly kill vegetative bacteria and fungi; – they do not reliably destroy endospores and viruses; – alcohols probably coagulate enzymes and proteins and damage lipid membranes; – proteins are more soluble and denature more easily in alcohol mixed with water and that is why aqueous solutions (70% ethanol) are more effective than pure alcohols. 2. Aldehydes: include both glutaraldehyde and formaldehyde. 3. Glutaraldehyde: – 2% solution of alkaline glutaraldehyde is one of the most widely used liquid sterilants for treating heat sensitive medical items; – it destroys all forms of microbial life including endospores and viruses. *Formaldehyde* is used as a gas or an aqueous 37% solution called formalin. It is an extremely effective germicide which kill all forms of life within minutes. It is used to kill bacteria and inactivates viruses for use as a vaccines. It is also used to preserve biological specimens. *Chlorhexidine* is extensively used in antiseptic products (skin creams, disinfectants and mouth wash). It has low toxicity and can kill a wide range of microbes including vegetative bacteria, fungi, and some enveloped viruses by reacting with proteins. *Ethylene oxide* is an extremely useful gaseous sterilizing agent; it destroys all microbes including endospores and viruses. A special chamber is used to sterilize items with ethylene oxide. It is carcinogenic in nature. *Halogens* include chlorine and iodine and are thought to work by oxidizing proteins and other cell components. *Chlorine* destroys all types of microorganisms and viruses. Chlorine releases compounds such as sodium hypochlorite is used

	to disinfect waste liquids, swimming pool water, instruments and surfaces, and at much lower concentration, it disinfects drinking water. Properly chlorinated drinking water contains ~ 0.5 ppm chlorine. Household bleach is 5.25% sodium hypochlorite. *Iodine* unlike chlorine, does not reliably kill endospores. Tincture of iodine or iodophores is used as disinfectants or antiseptics. Some *Pseudomonas* species survive in the concentrated solutions of iodophores
Antisepsis	*Antisepsis* is a process that prevents the development of sepsis. Antiseptics are applied to living tissue with the objective of preventing the multiplication of those vegetative microorganisms that are capable of producing sepsis. Because they are applied to living tissue, antiseptics are often milder agents than disinfectants. Antiseptics are expected to have at least a stasis effect on the microorganisms. In practice the term *antisepticize* is especially used in situations in which antiseptics are applied to cuts, abrasions, or lacerations, and to tissues before and after surgery

PHYSIOLOGY OF BACTERIA. BACTERIA NUTRITION AND RESPIRATION. NUTRIENT MEDIA. ISOLATION OF AEROBIC BACTERIA PURE CULTURE (STAGE 1)

Theme topicality. Bacteriological method of diagnosis is the main method of microbiological diagnostics of infectious diseases. It is important to every experienced physician to know how to conduct it, beginning with the selection of material for inoculation, and to evaluate the results of it.

Primary objective: to be able to cultivate bacteria on nutrient media, to isolate a pure culture and evaluate the results of identification tests.

QUESTIONS FOR DISCUSSION

Fig. 37.

1. Aims and methods of bacteria culturing.
2. Rules of work with bacterial cultures.
3. Nutrition of bacteria. Classification of the bacteria accoding to the types of nutrition.
4. Respiration of bacteria. Aerobes, anaerobes, microaerophiles.
5. Mechanisms of nutrients transmission in the bacterial cell.
6. Nutrient media, the classification according to the purpose and requirements.
7. Method of pure cultures isolation based on the biological principle.
8. Nutrient medium used for cultivation of anaerobic bacteria.
9. Stages of pure aerobic bacteria cultures isolation – stage 1.

PROCEDURE OF PRACTICAL WORK

Task 1. Study and sketch demonstration.

Culture media

MacConkey agar

Much of the study of microbiology depends on the ability to grow and maintain microorganisms in the laboratory, and this is possible only if suitable culture media are available. A culture medium is a solid or liquid preparation used to grow, transport, and store microorganisms. To be effective, the medium must contain all the nutrients the microorganism requires for growth.

Specialized media are essential in the isolation and identification of microorganisms, the testing of antibiotic sensitivities, water and food analysis, industrial microbiology, and other activities.

Although all microorganisms need sources of energy, carbon, nitrogen, phosphorus, sulphur, and various minerals, the precise composition of a satisfactory medium will depend on the species one is trying to cultivate because nutritional requirements vary so greatly.

Knowledge of a microorganism's normal habitat often is useful in selecting an appropriate culture medium because its nutrient requirements reflect its natural surroundings. Frequently a medium is used to select and grow specific microorganisms or to help identify a particular species. In such cases the function of the medium also will determine its composition.

Synthetic media

Some microorganisms, particularly photolithotrophic autotrophs such as cyanobacteria and eucaryotic algae, can be grown on relatively simple media containing CO_2 as a carbon source (often added as sodium carbonate or bicarbonate), nitrate or ammonia as a nitrogen source, sulfate, phosphate, and a variety of minerals.

Such a medium in which all components are known is a defined medium or synthetic medium**.**

Many chemoorganotrophic heterotrophs also can be grown in defined media with glucose as a carbon source and an ammonium salt as a nitrogen source.

Fig. 38.

Not all defined media are as simple but may be constructed from dozens of components. Defined media are used widely in research, as it is often desirable to know what the experimental microorganism is metabolizing.

Complex media

Media that contain some ingredients of unknown chemical composition are complex media. Such media are very useful, as a single complex medium may be sufficiently rich and complete to meet the nutritional

requirements of many different microorganisms.

In addition, complex media often are needed because the nutritional requirements of a particular microorganism are unknown, and thus a defined medium cannot be constructed. This is the situation with many fastidious bacteria, some of which may even require a medium containing blood or serum.

Complex media contain undefined components like peptones, meat extract, and yeast extract.

Peptones are protein hydrolysates prepared by partial proteolytic digestion of meat, casein, soya meal, gelatin, and other protein sources.

They serve as sources of carbon, energy, and nitrogen. Beef extract and yeast extract are aqueous extracts of lean beef and brewer's yeast, respectively. Beef extract contains amino acids, peptides, nucleotides, organic acids, vitamins, and minerals. Yeast extract is an excellent source of B vitamins as well as nitrogen and carbon compounds. Three commonly used complex media are nutrient broth, tryptic soy broth, and MacConkey agar.

If a solid medium is needed for surface cultivation of microorganisms, liquid media can be solidified with the addition of 1.0 to 2.0% agar; most commonly 1.5% is used. Agar is a sulfated polymer composed mainly of D-galactose, 3,6-anhydro-L-galactose, and D-glucuronic acid. It usually is extracted from red algae.

Agar is well suited as a solidifying agent because after it has been melted in boiling water, it can be cooled to about 40 to 42 °C before hardening and will not melt again until the temperature rises to about 80 to 90 °C.

Agar is also an excellent hardening agent because most microorganisms cannot degrade it.

Other solidifying agents are sometimes employed.

For example, silica gel is used to grow autotrophic bacteria on solid media in the absence of organic substances and to determine carbon sources for heterotrophic bacteria by supplementing the medium with various organic compounds.

Types of media

Media such as tryptic soy broth and tryptic soy agar are called general purpose media because they support the growth of many microorganisms.

Blood and other special nutrients may be added to general purpose media to encourage the growth of fastidious heterotrophs. These specially fortified media (e.g., blood agar) are called enriched media.

Selective media favor the growth of particular microorganisms. Bile salts or dyes like basic fuchsin and crystal violet favor the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria without affecting gram-negative organisms.

Endo agar, eosin methylene blue agar, and MacConkey agar, three media widely used for the detection of *E. coli* and related bacteria in water supplies and else where, contain dyes that suppress gram-positive bacterial growth.

MacConkey agar also contains bile salts. Bacteria also may be selected by incubation with nutrients that they specifically can use. A medium containing only cellulose as a carbon and energy source is quite effective in the isolation of cellulose-digesting bacteria. The possibilities for selection are endless, and there are dozens of special selective media in use.

Differential media are media that distinguish between different groups of bacteria and even permit tentative identification of microorganisms based on their biological characteristics.

Blood agar is both a differential medium and an enriched one. It distinguishes between haemolytic and nonhaemolytic bacteria.

Haemolytic bacteria (e.g., many streptococci and staphylococci isolated from throats) produce clear zones around their colonies because of red blood cell destruction.

MacConkey agar is both differential and selective. Since it contains lactose and neutral red dye, lactose-fermenting colonies appear pink to red in colour and are easily distinguished from colonies of nonfermenters.

Thermostat is a device in which stable temperature is supported by termoregulator. In fact, temperature is the factor of bacteria growth.

Environmental temperature profoundly affects microorganisms, like all other organisms. Indeed, microorganisms are particularly susceptible because they are usually unicellular and their temperature varies with that of the external environment. For these reasons, microbial cell temperature directly reflects that of the cell’s surroundings.

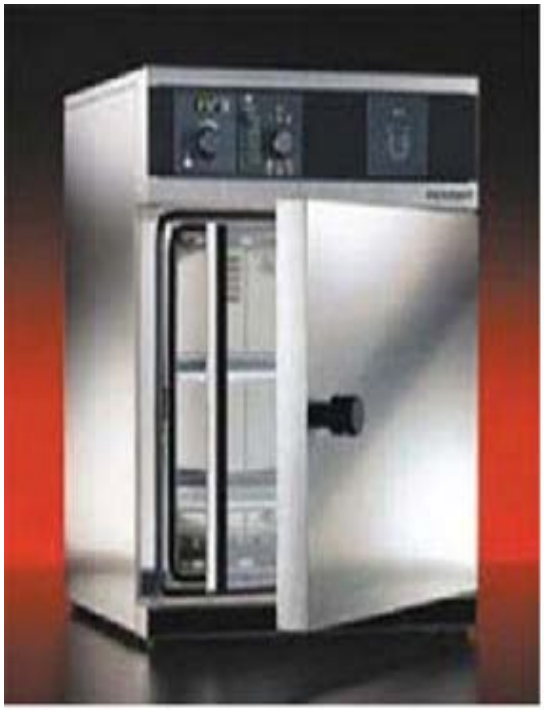


Fig. 39.

The most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme-catalyzed reactions.

At low temperatures a temperature rise increases the growth rate because the velocity of an enzyme-catalyzed reaction, like that of any chemical reaction, will roughly double for every 10°C rise in temperature.

Because the rate of each reaction increases, metabolism as a whole is more active at higher temperatures, and the microorganism grows faster.

Beyond a certain point further increases actually slow growth, and sufficiently high temperatures are lethal.

High temperatures damage microorganisms by denaturing enzymes, transport carriers, and other proteins.

Microbial membranes are also disrupted by temperature extremes; the lipid bilayer simply melts and disintegrates. Thus, although functional enzymes operate more rapidly at higher temperatures, the microorganism may be damaged to such an extent that growth is inhibited because the damage cannot be repaired. At very low temperatures, membranes solidify and enzymes don’t work rapidly. In summary, when organisms are above the optimum temperature, both function and cell structures are affected.

If temperatures are very low, function is affected but not necessarily cell chemical composition and structure.

Because of these opposing temperature influences, microbial growth has fairly characteristic temperature dependence with distinct cardinal temperatures - minimum, optimum, and maximum growth temperatures. Although the shape of the temperature dependence curve can vary, the temperature optimum is always closer to the maximum than to the minimum.

The cardinal temperatures for a particular species are not rigidly fixed but often depend to some extent on other environmental factors such as pH and the available nutrients.

For example, *Crithidia fasciculata,* a flagellated protozoan living in the gut of mosquitos, will grow in a simple medium at 22 – 27 °C.

However, it cannot be cultured at 33 – 34 °C without the addition of extra metals, amino acids, vitamins, and lipids. The cardinal temperatures vary greatly between microorganisms.

Optima normally range from 0°C to as high as 75 °C, whereas microbial growth occurs at temperatures extending from -20 °C to over 100 °C.

The major factor determining this growth range seems to be water. Even at the most extreme temperatures, microorganisms need liquid water to grow. The growth temperature range for a particular microorganism usually spans about 30 degrees. Some species (e.g., *Neisseria gonorrhoeae*) have a small range; others, like *Enterococcus faecalis,* will grow over a wide range of temperatures.

The major microbial groups differ from one another regarding their maximum growth temperature.

The upper limit for protozoa is about 50 °C. Some algae and fungi can grow at temperatures as high as 55 to 60 °C. Procaryotes have been found growing at or close to 100 °C, the boiling point of water at sea level. Recently strains growing at even higher temperatures have been discovered.

Clearly, procaryotic organisms can grow at much higher temperatures than eucaryotes.

It has been suggested that eucaryotes are not able to manufacture organellar membranes that are stable and functional at temperatures above 60 °C.

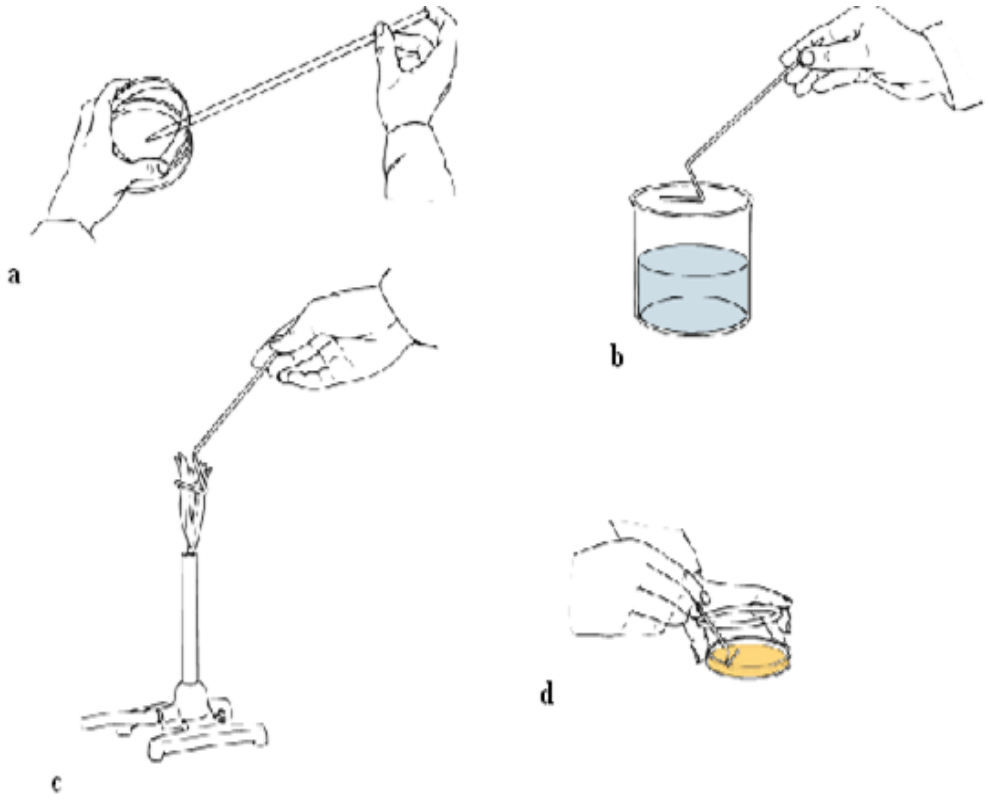
The photosynthetic apparatus also appears to be relatively unstable because photosynthetic organisms are not found growing at very high temperatures.

Task 2. Perform the first stage of pure culture isolation of *Escherichia coli* from bacterial mixed culture.

For the performance the stage 1 of pure culture isolation of *E. coli* from bacterial mixed culture, it is necessary to:

1) prepare a smear of the investigated material and stain it by Gram's method;

2) dilute the investigated material in sterile NaCl solution and inoculate into the Petri dish with Endo medium .



Procedure of material inoculation into Endo agar (task 2)

1. With a wax pencil, label the bottom of the agar medium plates with the name of the bacterium to be inoculated, your name, and date.
2. Pipette 0.1 ml of the respective bacterial mixculture onto the centre of a tryptic agar plate (a).
3. Dip the L-shaped glass rod into a beaker of ethanol (b) and then tap the rod on the side of the beaker to remove any excess ethanol.
4. Briefly pass the ethanol-soaked spreader through the flame to burn off the alcohol (c), and allow it to cool inside the lid of a sterile petri plate.
5. Spread the bacterial sample evenly over the agar surface with the sterilized spreader (d), making sure the entire surface of the plate has been covered. Also make sure you do not touch the edge of the plate.
6. Immerse the spreader in ethanol, tap on the side of the beaker to remove any excess ethanol, and reflame.
7. Repeat the procedure to inoculate the remaining two plates.
8. Invert the plates and incubate for 24 to 48 hours at 37 °C.
9. After incubation, measure some representative colonies and carefully observe their morphology.

Task 3. Perform the first stage of pure culture isolation of *staphylococcus* from the nose.

For the performance of the first stage of pure culture isolation of staphylococcus from the nose, it is necessary to:

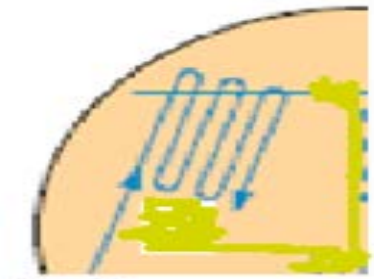


Fig. 41.

- take material with the mucous membrane of the nose with a help of wadding sterile tampon;
- paste the test tube with sterile cotton swab after taking a swab material with the material again in a test tube;
- take a test tube with swab in the left hand, clamp between index and middle fingers, so that its lower end freely lying on the thumb (left);
- remove the tampon with the right arm, left open the Petri dish with

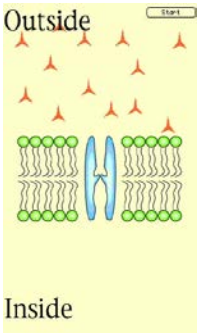
- EYA light and zigzag movements;
- make the inoculation on its surface do not damaging the medium;
- after inoculation the Petri dish is to be closed;
- sign your sector and put in a thermostat;
- slut the used tampon in place that will be autoclaving.

1 stage (1st day

Scheme of *Staphylococcus aureus* isolation in pure aerobic culture

Physiology of bacteria. Bacteria nutrition and respiration. Nutrient media. Isolation of pure cultures of aerobic bacteria (stage 1)

Notion	Definition/explanation
Metabolism	Metabolism is the total of all chemical reactions occurred in the cell. Metabolism may be divided into major parts. In *catabolism* larger and more complex molecules are broken down into smaller, simpler molecules with the release of energy. The bacterial cell obtains the energy for biochemical reaction due catabolism (energy-generating or energy-yielding process). *Anabolism* is the synthesis of complex molecules from simpler ones with the input of energy (energy-requiring process)
Pure culture	Pure culture is a population of cells arising from a single cell. Can be accomplished from mixtures by a variety of procedures, including streak plates and pour plates
Microbial nutrition	Nutrients are important to obtain energy and to construct new cellular components. Environmental factors such as temperature, oxygen levels, and water are important in cultivation of microorganisms. **Macroelements:** - are required by microorganisms in large amounts;
	- constitute 95% of cell dry weight; - C, O, H, N, S, P are components of carbohydrates, lipids, proteins, and nucleic acids; - K, Ca, Mg, Fe exist in the cell as cations and play a variety of roles (e.g., K^+ is required for activity of many enzymes including some involved in protein synthesis). **Microelements:** - trace elements or micronutrients (Mn, Zn, Co, Mo, Ni, Cu) are required in trace amounts by most cells; - are often adequately supplied in the water used to prepare the media. **Growth factors:** - organic compounds are required by the cell because they

	are essential cell components (or precursors of these components) that the cell cannot synthesize; - there are 3 major classes of growth factors: amino acids (are needed for protein synthesis); purines and pyrimidines (are needed for nucleic acid synthesis); purines and pyrimidines (are needed for nucleic acid synthesis)
Nutritional types of microorganisms	**Carbon source:** - *autotrophs* use carbon dioxide as their sole or principal carbon source; - *heterotrophs* use reduced, preformed organic molecules (usually from other organisms) as carbon sources. **Energy source:** - *phototrophs* use light as their energy source; - *chemotrophs* obtain energy from the oxidation of organic or inorganic compounds
Uptake of nutrients by the cell	The uptake mechanisms of microbial cells are specific.  Microorganisms make use of several different transport mechanisms. **Passive diffusion:** - a phenomenon in which molecules move from an area of high concentration to an area of low concentration because of random thermal agitation. - requires a large concentration gradient for significant levels of uptake.  - limited to only a few small useful molecules (e.g., glycerol,H_2O, O_2, and CO_2). **Facilitated diffusion:** - a process that involves a carrier molecule (permease) to increase the rate of diffusion; net effect is limited to movement from an area of higher concentration to an area of lower concentration; - requires a smaller concentration gradient than passive diffusion; - the rate plateaus when the carrier becomes saturated (i.e.,

	when it is binding and transporting molecules as rapidly as possible); - generally more important in eucaryotes rather than procaryotes. **Active transport:** - a process in which metabolic energy is used to move molecules to the cell interior where the solute concentration is already higher (i.e., it runs against the concentration gradient); - requires an expenditure of metabolic energy, from ATP hydrolysis;

Table 1.5.1 continuation

Notion	Definition/explanation
	- can concentrate molecules inside the cell even when the concentration inside the cell is already higher than that outside the cell. There are 2 types of active transport: 1. *Symport* is the linked transport of two substances in the same direction. 2. *Antiport* is the linked transport of two substances in opposite directions. **Group translocation:** - a process in which molecules are modified as they are transported across the membrane
Culture media	Much of microbiology depends on the ability to grow and maintain microorganisms in the laboratory. This is possible only if suitable culture media is available. Media can be defined (synthetic) or complex, and liquid or solid.
Classification of culture media	Based on the consistency: - liquid – peptone water, nutrient broth; - semisolid – nutrient agar stabs; - solid – blood agar, serum agar. Based on oxygen requirement: - aerobic medium; - anaerobic medium
Synthetic	Synthetic (defined) media are media in which all components and their concentrations are known.

(defined) media	1. Peptones: protein hydrolysates prepared by partial proteolytic digestion of various protein sources. 2. Extracts: aqueous extracts, usually of beef or yeast
Liquid media	Liquid media: the easiest to prepare and use. Good for growing quantities of microbes needed for analysis or experiments. Unless inoculated with pure culture, cannot separate different organisms
Solid media	Solid media is needed for surface cultivation of microorganisms
Agar	Agar is a sulphated polymer, extracted from red algae
Aerobic media	Simple medium. Complex medium. Enriched medium. Differential medium. Enrichment medium. Selective medium. Transport medium
Enriched media	Enriched media are general purpose media supplemented by blood or other special nutrients to encourage the growth of fastidious heterotrophs; (fastidious = having complicated nutritional requirements)
Selective media	Inhibits the growth of some bacteria while selecting for the growth of others. Example: brilliant green agar – dyes inhibit the growth of gram-positive bacteria; selects for gram-negative bacteria
Differential media	Differential media distinguish between different groups of bacteria on the basis of their biological characteristics. They cause observable change in medium when biochemical reaction occurs. For example: MacConkey agar has color indicator that distinguishes presence of acid. Bacteria that ferment a particular sugar (e.g., glucose in culture media) will produce acid wastes on plates, turn pH indicator red. Bacteria that cannot ferment the same sugar

Table 1.5.1 continuation

Notion	**Definition/explanation**

	will grow but not affect pH, so colonies remain white
Complex media	Enriched media: blood agar (nutrient agar + 5 to 10% sheep blood). Melt the sterile nutrient agar by steaming, cool to 45 °C. Add the blood aseptically with constant shaking. Mix the blood with molten nutrient agar thoroughly but gently avoiding froth formation. Immediately pour in to the Petri dishes or tubes and allow setting. Use: cultivate all the fastidious organisms
Special culture techniques	1. Anaerobic bacteria; a) reducing media; b) anaerobic container; c) agar stab; d) agar shake. 2. Microaerophilic bacteria: - grow best under reduced O_2 levels and increased CO_2 levels; - normal atmosphere 21% O_2, 03% CO_2
Transport media	Are used in case of delicate organisms whenever there is a delay in the transportation of the specimen to the lab. To maintain viability of them and to prevent the multiplication of non-pathogenic bacteria: - Stuart's medium – gonococci; - Cary-Blair's medium – *V. cholerae;* - V-R medium – *V. cholerae*
Sterilization of culture media	Media are sterilized in the autoclave at 121 °C for 15 min under 15 lbs of pressure. Heat-labile substances like serum and sugar solutions must be sterilized by free-steam or filtration Egg containing media – Lowenstein-Jensen's medium, Loeffler's serum slope by inspissation. Discarded culture plates are to be sterilized by autoclaving prior to washing
Classification of the microorganisms based on their	1. **Psychrophiles** grow well at 0 °C and have an optimum growth temperature of 15 °C or lower; the maximum is around 20 °C. They are readily isolated from Arctic and Antarctic habitats; because 90% of the ocean is 5 °C or colder, it

temperature ranges for growth	constitutes an enormous habitat for psychrophiles. The psychrophilic alga *Chlamydomonas nivalis* can actually turn a snowfield or glacier pink with its bright red spores. Psychrophiles are widespread among bacterial taxa and found in such genera as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium,* and *Shewanella.* The psychrophilic archaeon *Methanogenium* has recently been isolated from Ace Lake in Antarctica. Psychrophilic microorganisms have adapted to their environment in several ways. Their enzymes, transport systems, and protein synthetic mechanisms function well at low temperatures. The cell membranes of psychrophilic microorganisms have high levels of unsaturated fatty acids and remain semifluid when cold. Indeed, many psychrophiles begin to leak cellular constituents at temperatures higher than 20 °C because of cell membrane disruption. 2. Many species can grow at 0 to 7 °C even though they have optima between 20 and 30 °C, and maxima at about 35 °C. These are called psychrotrophs or facultative psychrophiles**.** Psychrotrophic bacteria and fungi are major factors in the spoilage of refrigerated foods

Table 1.5.1 continuation

Notion	**Definition/explanation**
	3. Mesophiles are microorganisms with growth optima around 20 to 45 °C; they often have a temperature minimum of 15 to 20 °C. Their maximum is about 45 °C or lower. Most microorganisms probably fall within this category. Almost all human pathogens are mesophiles, as might be expected since their environment is a fairly constant 37 °C. 4. Some microorganisms are thermophiles; they can grow at temperatures of 55 °C or higher. Their growth minimum is usually around 45 °C and they often have optima between 55 and 65 °C. The vast majority are procaryotes although a few algae and fungi are thermophilic. These organisms flourish in many habitats including composts, self-heating hay stacks, hot water lines, and hot

	springs. Thermophiles differ from mesophiles in having much more heat-stable enzymes and protein synthesis systems able to function at high temperatures. Their membrane lipids are also more saturated than those of mesophiles and have higher melting points; therefore thermophile membranes remain intact at higher temperatures. 5. As mentioned previously, a few thermophiles can grow at 90 °C or above and some have maxima above 100 °C. Procaryotes that have growth optima between 80 °C and about 113 °C are called hyperthermophiles**.** They usually do not grow well below 55 °C. *Pyrococcus abyssi* and *Pyrodictium occultum* are examples of marine hyperthermophiles found in hot areas of the seafloor
Several methods for isolation of microorganisms in pure culture	**1. Plating:** a) *Spread plate*. A small volume of dilute microbial mixture containing is transferred to the centre of an agar plate and spread evenly over the surface with a sterile bent-glass rod. The dispersed cells develop into isolated colonies. b) *Streak plate* (fig. 44)**.** The original suspension can be streaked on an agar plate with a wire loop. As the streaking continues, fewer and fewer cells are left on the loop, and finally the loop may deposit single cells on the agar. c) *Pour plate* (fig.43)**.** In the pour-plate method, a suspension of cells is mixed with melted agar at 50 °C and poured into a Petri dish. When the agar solidifies, the cells are immobilized in the agar and grow into colonies. **2. Dilution.** A much less reliable method is that of extinction dilution. The suspension is serially diluted and samples of each dilution are plated. If only a few samples of a particular dilution exhibit growth, it is presumed that some of these cultures started from single cells. This method is not used unless plating is for some reason impossible. An undesirable feature of this method is that it can only be used to isolate the predominant type of organism in a mixed population

The chart of bacteriological method of diagnostics of infectious diseases

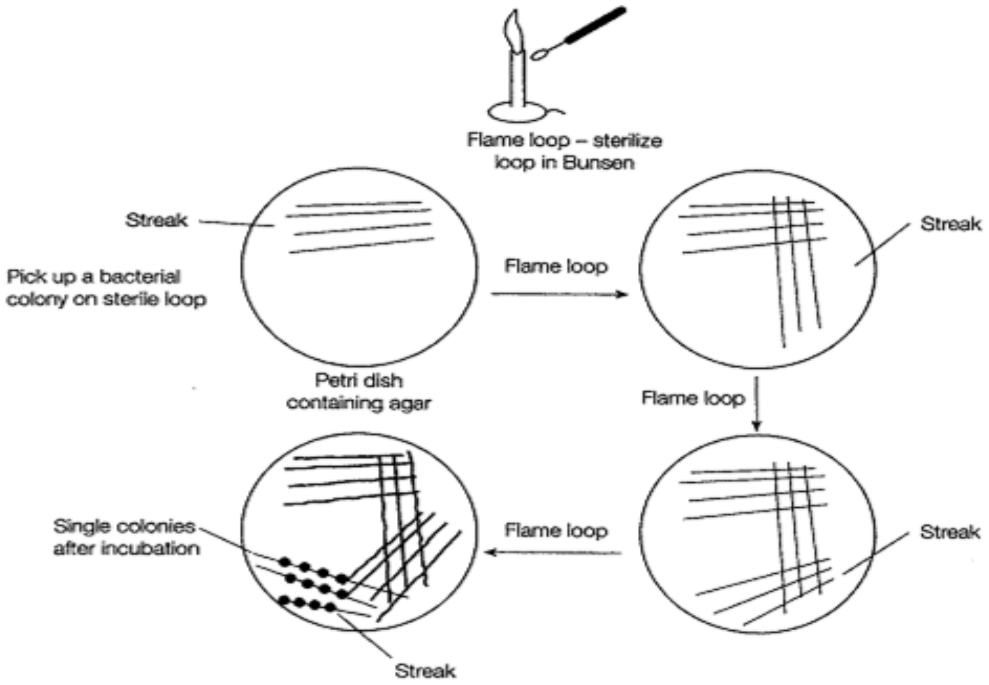
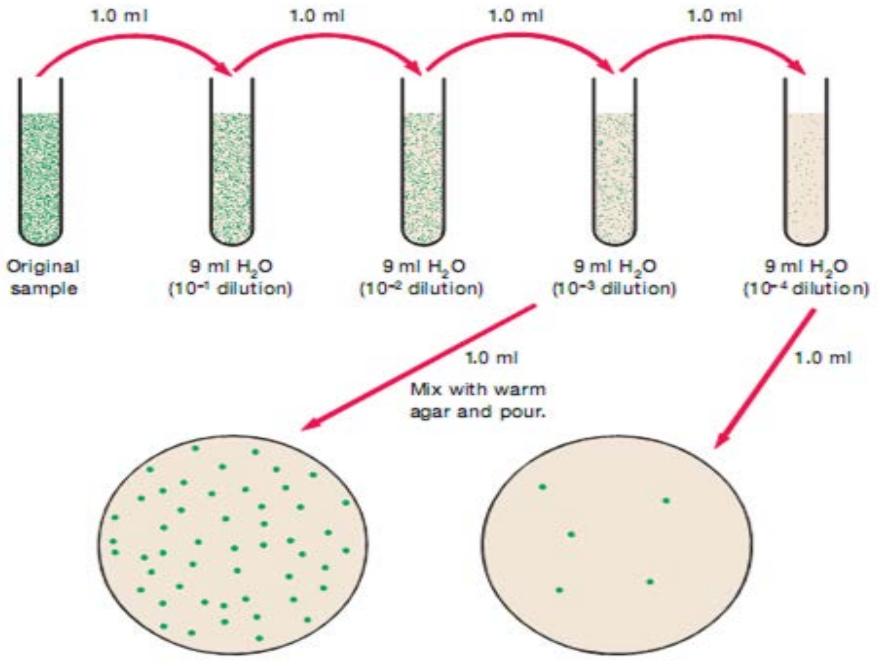


Figure 1.5.6 – The streak plate technique



The original sample is diluted several times to thin out the population sufficiently. The most diluted samples are then mixed with warm agar and poured into Petri dishes. Isolated cells grow into colonies and can be used to establish pure cultures.

The surface colonies are circular; subsurface colonies would be lenticular or lens shaped.

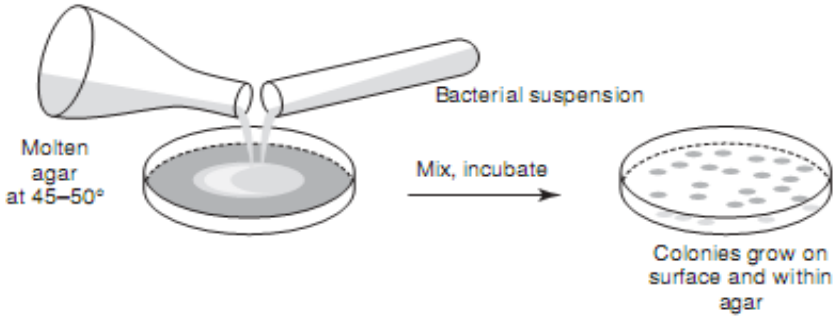


Figure 1.5.7 – The pour-plate technique

GROWTH AND REPRODUCTION OF BACTERIA. PURE CULTURES ISOLATION AEROBIC BACTERIA (stage 2). METHOD OF CULTURING. ENZYMES.

Theme topicality. In laboratory practice one has to work with microorganisms grown on nutrient medium for isolation (accumulation) and identification of pure culture.

Primary objective: to be able to determine the morphological, tinctorial, cultural, and biochemical properties of bacteria nutrient media.

QUESTIONS FOR DISCUSSION

1. Principle of microorganisms cultivation on nutrient media.
2. Growth and reproduction of microorganisms. Phases of bacterial populations, development.
3. Physical and environmental requirements for microbial growth.
4. The terms “colony” and “culture”. Their definitions.
5. Cultural properties of bacteria. Characteristics of colonies (growth in liquid and solid culture media).

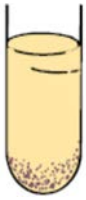
6. The second stage of pure culture isolation: the main purpose and procedure.
7. Enzymes of bacteria. Classification of enzymes.

PROCEDURE OF PRACTICAL WORK

Task 1. Study and sketch demonstration.

Staphylococcus forms a yellow pigment, medium-sized colonies, with even edges. Collibacillus forms colourless, turbid, brilliant colonies with even edges.

Figure 1.6.1 – Growth of staphylococcus and collibacillus on nutrient agar



Streptococcus on sugar broth forms bottom or parietal growth. Thus peptone broth remains transparent.

Figure 1.6.2 – Growth of streptococcus on sugar broth

Vibrio cholerae forms tender blue film on nutrient broth.

Figure 1.6.3 – Growth of *Vibrio cholerae* on nutrient broth

film

Sporeforming bacteria form rough on nutrient broth.

Figure 1.6.4 – Growth of spore-forming bacteria on nutrient broth
Staphylococcus forms diffusion growth on nutrient broth.

Figure 1.6.5 – Growth of *S. aureus* on nutrient broth

Bacterial growth on solid media and description of their cultural properties.

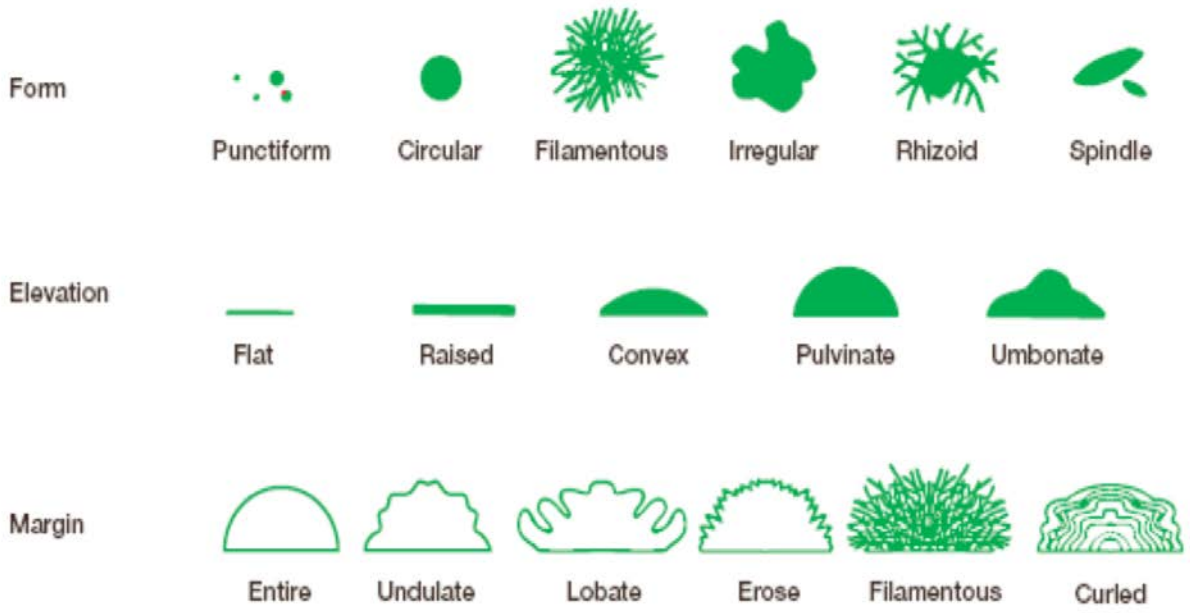


Figure 1.6.6 – Bacterial colony morphology

Variations in bacterial colony morphology are seen with the naked eye.

The general form of the colony and the shape of the edge or margin can be determined by looking down at the top of the colony. The nature of colony elevation is apparent when viewed from the side as the plate is held at eye level.

Task 2. Perform the 2nd stage of pure culture isolation of *E. coli* from mixed culture.

For performance of stage 2 one should:

1. Describe cultural properties of colonies which grew on Endo medium. Students are to write down these properties in the protocol.
2. Prepare smears of different colonies, stain by Gram's method and perform microscopic examination. Write down the obtained results on morphological and staining properties in to the protocol.
3. Reinoculate the part of colony of which the specimen was prepared on slant beef-extract agar.

Procedure of inoculation

A. Transfer of a slant culture to a nutrient broth

1. The procedure will be demonstrated. Watch carefully and then do it yourself, following the directions.
2. Take up the inoculating loop by the handle and hold it as you would a pencil, loop down. Hold the wire in the flame of the spirit lamp. Remove loop and hold it steady a few moments until cool. Do not wave it around, put it down, or touch it to anything.
3. Pick up the slant culture of *Escherichia coli* with your left hand. Still holding the loop likes a pencil, but more horizontally, in your right hand, use the little finger of the loop hand to remove the closure (cotton plug, slip-on, or screw cap) of the culture tube. Keep your little finger curled around this closure when it is free do not place it on the table.
4. Insert the loop into the open tube (holding both horizontally). Touch the loop (not the handle) to the growth on the slant and remove a loopful of culture. Don't dig the loop into the agar; merely scrape a small surface area gently.
5. Withdraw the loop slowly and steadily, being careful not to touch it to the mouth of the tube. Keep it steady, and do not touch it to anything (it's loaded) while you replace the tube closure and put the tube back in the rack.
6. Still holding the loop steady in one hand, use the other hand to pick up a tube of sterile nutrient broth from the rack. Now remove the tube closure, as you did before, with the little finger of the loop hand (don't wave or jar the loop). Insert the loop into the tube and down into the broth. Gently rub the loop against the wall of the tube (don't agitate or splash the broth), making sure the liquid covers the area but does not touch the loop handle.

7. As you withdraw the loop, touch it to the inside wall of the tube (not the tube's mouth) to remove excess fluid from it. Pull it out without touching it again, replace the closure, and put the tube back in the rack.

8. Now carefully sterilize the loop. If you are using a Bunsen burner, hold it first in the coolest part of the flame (yellow), then in the hot blue cone until it glows. Be sure all of the wire is sterilized, but do not burn the handle. When the wire has cooled, the loop can be placed on the bench top.

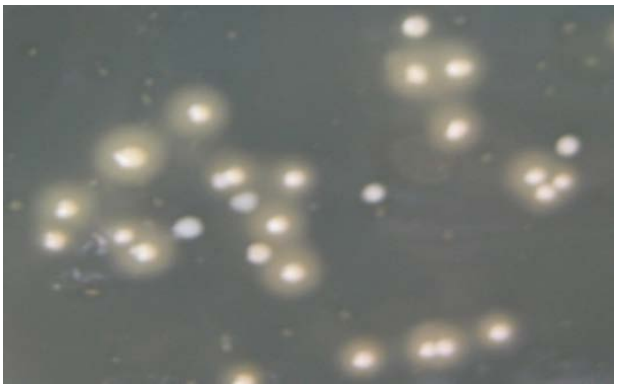
9. Label the tube you have just inoculated with your name, the name of the organism, and the date.

B. Transfer of a slant culture to a nutrient agar slant

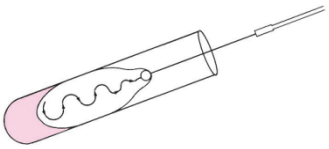
1. Start again with sterilizing the loop.

2. Pick up the slant culture of *E. coli*, open it, and take up some growth on the sterile loop.

3. Recap the culture tube carefully and replace it in the rack. Pick up and open a sterile nutrient agar slant (keep the charged loop steady meantime).



4. Introduce the charged loop into the fresh tube of agar, and without touching any surface, pass it down the tube to the deep end of the slant. Streak the agar slant by lightly touching the loop to the surface of the agar, swishing it back and forth two or three times (don't dig up the agar), then zigzagging it upward to the top of the slant. Lift the loop from the agar surface and withdraw it from the tube without touching the tube surfaces.



5. Close and replace the inoculated tube in the rack; then sterilize the loop as before.

6. Label the freshly inoculated tube with your name, the name of the organism, and the date.

Figure 1.6.7 – Growth of the staphylococcus on egg yolk agar

C. Incubation of freshly inoculated cultures

1. Make certain all the broths (4) and slants (4) you have inoculated are properly and fully labeled.

2. Place your transferred cultures in an assigned rack in the incubator. The incubator temperature should be 35 to 37 °C.

Table 1.6.1 – Cultural properties of the bacteria

1. Shape (circular, irregular, radiate or rhizoid)
2. Size (small – 1–2 mm, intermediate – 2–4 mm, large – 4–5 mm, dwarf-less then 1 mm)
3. Margins or edges (entire, crenate, undulate, fimbriated or curled)
4. Surfaces (smooth – S-form, rough or coarsely granular – R-form, wavy, glistening)
5. Elevation (effuse, elevated, convex, low convex, convex papillate, concave, umbonate or cate)
6. Colour (pigments) (white, yellow, gold, lemon, orange, green, blue, grey, black, brown, red)
7. Opacity (opaque, translucent or transparent)
8. Consistency (membranous, friable, butyrous, viscid, soft or hard)

Task 3. Perform the 2nd stage of pure culture isolation of *S. aureus* from the nose.

For performance of stage 2 one should:

1. Describe cultural properties of colonies which grown on egg yolk agar. Students are to write down these properties in the protocol.

2. Prepare smears of different colonies, stain them by Gram's method, and perform microscopic examination. Write down the obtained results on morphological and staining properties in to the protocol.

3. Reinoculate the part of colony of which the specimen was prepared on the slant nutrient agar (procedure is present in task 3).

If staphylococcus is pathogenic, round colony in this zone is present, because pathogenic staphylococci produce the enzyme of pathogenicity and it destroys lecithin in the nutrient media.

Table 1.6.1 – Growth and reproduction of bacteria. Pure culture isolation aerobic bacteria (stage 2). Method of culturing. Enzymes

Notion	Definition/explanation
Reproduction and growth of microorganisms	For bacterial population reproduction is the increase in the number of individuals per unit volume. The growth of microorganisms represents the increase of the mass of bacterial cytoplasm as a result of the synthesis of cellular material

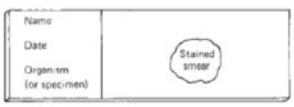
Reproduce of bacteria	1. Binary fission or simple transverse division or vegetative reproduction (is more common way of reproduction). 2. Budding by means of the cleavage of segmented filaments. 3. Sporulation (feature of fungi and actynomycetes reproduction)
Overview of aerobic respiration	1. Glycolysis – glucose (6C) is oxidized and split into 2 molecules of pyruvic acid (3C). 2. TCA processes pyruvic acid and generates 3 CO_2 molecules. 3. Electron transport chain accepts electrons NADH and FADH, generates energy through sequential redox reactions called oxidative phosphorylation
Isolation of microbes from clinical material	1. Microscopic examination: Gram staining, acid fast stain, dark field microscopy, phases contrast. 2. Culture: use streak plate to culture sample on appropriate media to get pure culture. Use in addition selective media for isolation of the suspected organism. 3. Identification: use staining, differential media, serology and or gene probe for identification of the isolated microbe. 4. In vitro antibiotic sensitivity test. 5. Epidemiology in case of outbreak to determine the source of infection
Clone	A race of cells derived from a single ancestral cell and sharing a single function
Colony	A number of microorganisms living or multiplying together on solid culture media in the result of multiplication of a single cell
Pure culture	Pure culture is a population of microorganism of the same species isolated on a nutrient medium
Characteristic of stroke culture	1. The degree of growth – scanty, moderate or profuse. 2. Their nature – discrete or confluent, filiform, spreading or rhizoid

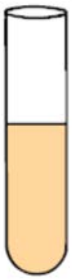
Table 1.6.1 continuation

Notion	Definition/explanation
	3. Their elevation, surface, edges, colour, structure, odour, emuisifiability, consistency and cha the medium are noted
The growth curve	1. Lag phase – “flat” period of adjustment, enlargement; little growth. 2. Exponential growth phase – a period of maximum growth last as long as cells have adequate nutrients and favorable environment. 3. Stationary phase – rate of cell growth equals rate of cell death cause by depleted nutrients and O_2, excretion of organic acids and pollutants. 4. Death phase – as limiting factors intensify, cells die exponentially in their own wastes
Cultural properties of bacteria on solid media	Cultural characteristics of bacteria mean the morphology their colonies and features of growth in a fluid media. While studying colonies on solid media, the following features are noted: shape: circular, irregular, or rhizoid; size in millimeters; elevation: effuse, elevated, convex, concave; margins – beveled or otherwise; surface – smooth, wavy, rough, granular, etc.;edges – entire, undulate, curled; colours; structure – opaque, translucent, transparent; consistency – friable, membranous or viscid; emulsifiability
Cultural properties of	In liquid nutrient media microbes grow producing a diffuse

bacteria on liquid media	suspension, film or precipitate visible to the naked eye
Classification of enzymes	1. Exoenzymes are transported extracellularly, where they break down large food molecules or harmful chemicals; cellulase, amylase, penicillinase. 2. Endoenzymes are retained intracellularly and function there. 3. Constitutive enzymes are always present, they are produced in equal amounts or at equal rates, regardless of amount of substrate; enzymes are involved in glucose metabolism. 4. Induced enzymes are not constantly present, they are produced only when substrate is present; prevent cells from resources loss
Group of bacterial enzymes	1. Hydrolases which catalyze the breakdown of the link between the carbon and nitrogen atoms, between the oxygen and sulphur atoms, binding one molecule of water (esterases. glucosidases, proteases. amilases, nucleases, etc.). 2. Transferases perform catalysis by transferring certain radicals from one molecule to another (transglucosidases, transacylases. transaminases). 3. Oxidative enzymes (oxyreductases) which catalyze the oxidation-reduction processes (oxidases, dehydrogenases, peroxidases, catalases). 4. Isomerases and racemases play an important part in carbohydrate metabolism. They are found in most species of bacteria
Enzymes transfer reactions	1. Oxidation-reduction reactions are transfer of electrons. 2. Aminotransferases convert one type of amino acid to another by transferring an amino group. 3. Phosphotransferases transfer phosphate groups, involved in energy transfer. 4. Methyltransferases transfer methyl groups from one molecule to another. 5. Decarboxylases transfer carbon dioxide from organic acids

Sheme of pure culture isolation of aerobic bacteria's
Stage 1 of pure culture isolation of aerobic bacteria – 1st day





Stage 2 of pure culture isolation of aerobic bacteria – 2nd day

1.7. STAGES 3 AND 4 PURE CULTURE BACTERIA ISOLATION. ENZYMES OF BACTERIA AND THEIR VALUE FOR IDENTIFICATION OF MICROORGANISMS. ANTIBIOTIC THERAPY

Actuality of the theme. By the bacteriological methods of diagnosis stands pure culture of bacteria - pathogens of infectious diseases. For identification of pure culture of bacteria the study of enzymatic properties and pathogenicity factors selected pure culture is an important part of the bacteriologist. Very important in the isolation of microorganisms is to determine their sensitivity to antibiotics in order to choose the optimal drug for treatment.

Primary objectives: able to identify species and determine the sensitivity of pathogens to antibiotics.

QUESTIONS FOR DISCUSSION

1. Enzymes of bacteria, practical value.
2. Practical use of the fermentative properties of microorganisms.
3. Characteristic of differential diagnostic media for the determination of fermentation of the saccharolytic action of bacteria.
4. Identification of pure culture (morphological, tinctorial, cultural, biochemical, serological, biological) (stages 3 and 4 of bacteriological method).
5. Antibiotics: definition, classification.
6. Spectrum of action and efficacy of antibiotics (bacteriostasis, bactericidal activity, postantibiotic effect (L-forms)).

7. Mechanisms of action of antibiotics.

1. Side effects of antibiotics: toxic effects, allergic reactions, biological side effects.
2. The problem of resistance: definition, species, clinical and biochemical resistance.
3. Tests of antimicrobial drugs: sensitivity tests, disk diffusion, interpret action of the results.

PROCEDURE OF PRACTICAL WORK

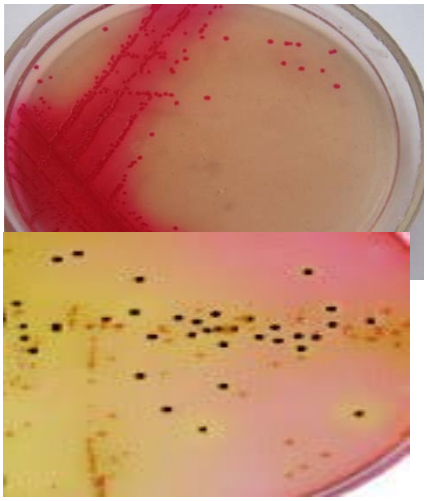
Task 1. Study and sketch demonstration.

Examine the growth of *E. coli* on Endo medium. Draw the Petri dish with growth of the culture

Endo medium consists of nutrient agar, lactose, and fuchsin which is decolourized by sodium sulphite. Freshly prepared medium is colourless (i.e., has the colour of agar). Colibacilli ferment lactose with the formation of aldehydes, fuchsin-sulphuric acid changes to aldehyde-sulphuric substance with the release of fuchsine that stains medium into bright red.

Therefore, on this medium *E. coli* form red (lactose-positive) colonies.

Figure 1.7.1 – Growth of *E. coli* on Endo medium.



Growth of *E. coli* and *S. typhi* on Ploskirev's medium.

Ploskirev's medium contains dry nutrient agar with lactose, different buffers, and other salts, bile salts, brilliant green and indicator neutral red. On this medium colibacilli grow very weakly because of inhibition of its viability by bile salts and brilliant green. This medium is employed for cultivating of pathogenic causative agents (dysentery, enteric fever).

Figure 1.7.2 – Growth of *E. coli* and *S. typhi* on Ploskirev's medium

Bacterial growth on Ressel medium. Describe the changes of media; make the conclusion about fermentative activity

Ressel medium consists of beef-extract agar, 0.1% solution of glucose, 1% solution of lactose, and indicator. It is spilled to the test tubes in such way that the lower part is the stab, and the upper part is the slant. Bacteria, that ferment both carbohydrates, change the colour of media both in the stab and the slant, and at gas formation in the stab the vesicles appear or the medium disrupts. Bacteria, that

ferment glucose to acid and don't ferment lactose, change the colour of medium only in stab, but not in the slant.

Blood agar is a special medium which ability of bacteria to produce hemolysin comes to light on. On blood agar, abundant growth of most staphylococcal species occurs within 18 to 24 hours. Only individual colonies should be picked for preliminary identification testing at this time. Since most species cannot be distiguished from each other in the basis of the colony morphology with a 24-hours incubation period, colonies should be allowed to grow for at least an additional 2 to 3 days before the primary isolation plate is confirmed for species or strain composition.

S. aureus on blood agar forms colonies round which the haemolysis (enlightenment, at the expense of destruction erythrocytes) is formed. It occurs at the expense of allocation by bacteria haemolysin. Ability of bacteria to allocate haemolysin is one of pathogenic signs.

At *S. epidermidis* there is no ability to synthesized haemolysins. Therefore round colonies there is no zone haemolysis.

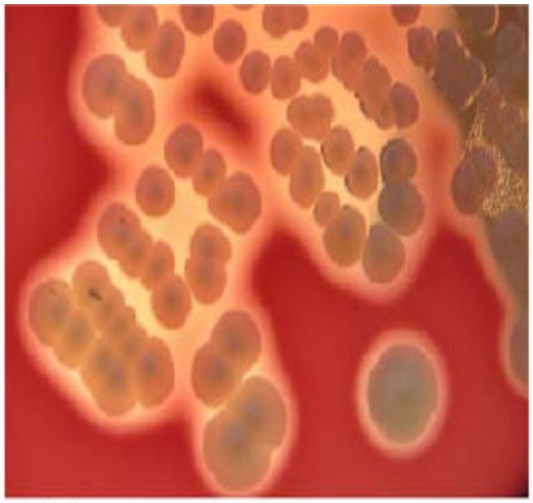


Fig. 58.

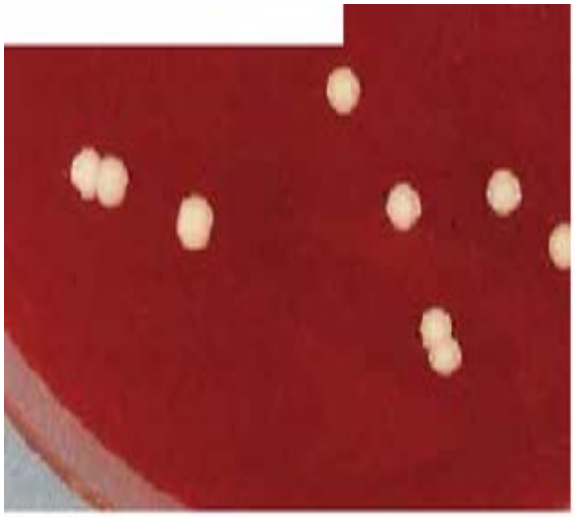
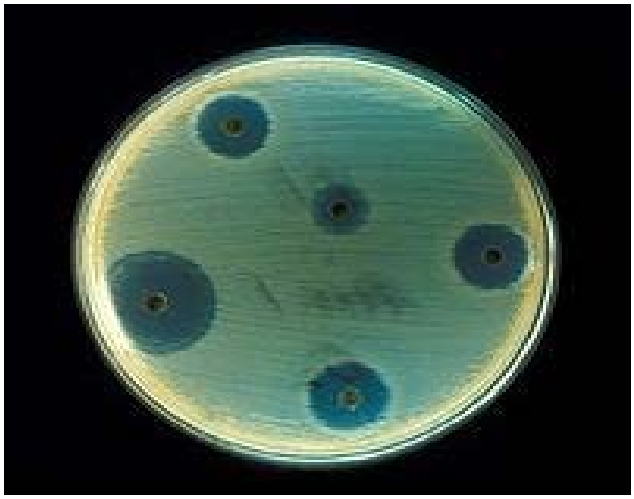


Fig. 59.

Sensitivity of staphylococci to antibiotics
by agar disk diffusion method. Draw the Petri dish with paper disks.



Disk method. Into sterile Petri dishes placed on the horizontal surface, pour 15 ml of solid nutrient medium. On the surface of solidified and slightly dried agar, pour 1 ml of suspension of 24–hour culture of the causative agent or. if no pure culture has been isolated, of the pathological material (pus, exudate) obtained for the study and diluted with isotonic saline. Spread uniformly over the agar surface the bacterial suspension, removing its remainder with a pasteur pipette.

Disks with antibiotics (5–6 disks per plate) are placed onto the surface of the inoculated plate (distance from its centre is 25 mm.

The plates are incubated at 37 °C for 16–18 h, after that the results of the test are read by measuring the zones of growth retardation of microorganisms around the disks, including the diameter of the disk itself. The size of the zones depends on die degree of sensitivity of the causative agent to a given antibiotic.

The strain is considered stable if the diameter of the zone is less than 10 mm, weakly sensitive if it averages 11–15 mm, and sensitive if it reaches 15–25 mm. Zones exceeding 25 mm in diameter indicate high sensitivity of the microorganism to the given antibiotic. Yet, this method cannot be considered strictly quantitative.

Task 2. Perform the 4th stage of pure culture isolation of *E. coli* from the mixed culture.

Third day. In the second phase was carried out planting colonies on the slant. This is done to the accumulation of a pure culture of bacteria. After 24-hour incubation using the culture which has grown on the agar slant prepare smears and stain them by the Gram method. Such characteristics as homogeneity of the growth, form, size, and staining of microorganisms permit definite conclusion as to purity of the culture. Checking of the purity of the grown culture on slant agar, making smear, staining by Gram method. Sketch in album.

Then sub-inoculating culture on Hiss medium, peptone water for determination of sugarlytic and peptolytic properties of bacteria; conduct posing a disk diffusion method to determine the sensitivity at antibiotics, ect.

Fourth day. For performance of the 4th stage one should:

1. Do the account of biochemical properties of the isolated pure culture on Hiss medium.
2. Exanime the catalase with a pure culture.

The activity of catalase

Many bacteria produce the catalase enzyme, which breaks down hydrogen peroxide, evolving oxygen. The simple test for catalase can be very useful in distinguishing between organism groups. Hydrogen peroxide can be added directly to a slant culture or to bacteria smeared on a clean glass slide. The test should not be performed with organisms growing on a blood-containing medium because catalase is found in red blood cells.

To observe bacterial catalase activity it is necessary to use:

- 3% hydrogen peroxide;
- capillary pipettes;
- pipette bulb or other aspiration devices;
- nutrient agar slant cultures of colibacteria;
- clean glass slides;
- china-marking pencil or marking pen.

Procedures

1. Divide a clean glass slide into two sections with your marking pen or pencil.
2. Sterilize the loop again and smear a small amount of the colibacteria culture on the right-hand side of the slide.
3. With the capillary pipette, place one drop of hydrogen peroxide over each smear. Be careful not to run the drops together.
4. Observe the fluid over the smears for the appearance of blebs. Record the results in the chart. Discard the slide in a jar of disinfectant.
5. Repeat the procedure with the colibacteria culture. Note whether oxygen is liberated and bubbling occurs.
6. Examine the sensitivity of colibacteria to antibiotics by agar disk diffusion method.
7. After accounting for all tests, the identification and antibiotikogramy write the conclusion of the microorganism species membership.

Task 3. Perform the 4th stage of pure culture isolation of *S.aureus* from nose.

For the performance of the 4th stage one should:

1. Describe cultural properties of colonies grown on blood agar. Students are to write down these properties in the protocol.

2. Prepare smears of different colonies on blood agar, stain them by Gram's method, and perform microscopic examination. Write down the obtained results on morphological and staining properties in to the protocol.

3. Study and sketch the reactions of plasma coagulase.

4. Study biochemical properties of the isolated pure culture on Hiss media with mannitol.

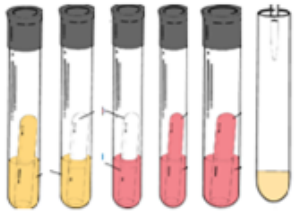
5. Examine the sensitivity of staphylococci to antibiotics by agar disk diffusion method.

6. Write a conclusion about pure culture.

1. Prepare smear, stain this preparate by Gram method for study of morphological and tinctorial properties and for study of this grow to forming one type of bacteria or non. If in preparate present only one morphological type G+ or G- bacteria's, are regrowth on: to supply tests for study pathogenic enzymes, for example plasmocoagulase, catalase, DNAse test. Registration of result.

2. For study haemolytic properties regrowth on blood agar – registration of result after incubation in thermostat at 37 °C for 18–24 h.

3. For study of biochemical properties, pure culture is reinoculated on Hiss media with another sugar or another differential diagnostic medium and peptone water for the study of proteolic enzymes – incubation in thermostat at 37 °C for 18–24 h.



Registration of biochemical properties on Hiss media

4. Supply test for sensitivity at antibiotics – incubation in thermostat at 37 °C for 18–24 h.

5. Study antigenic properties pure culture in agglutination test with specific agglutination serum

Identification of pure culture with using morphological, staining, pathogenic, antigenic (stages 2–3), and biochemical properties, bacteriophages and antibiotics sensitivity.

FINAL RESULT

1. 8 OBLIGATE ANAEROBIC BACTERIA. METHODS OF ANAEROBIC CULTURE ISOLATION. BIOLOGICAL METHOD OF INVESTIGATION

Theme topicality. Bacteriological and biological method of diagnosis is the main method of microbiological diagnosis of anaerobic infectious diseases, such as gas gangrene, and tetanus. Every experienced physician must be able to know how to conduct it beginning with the selection of material for staining, and to evaluate the obtained results.

Primary objective: to be able to cultivate anaerobic bacteria on nutrient media, isolate pure culture, and evaluate the results of identification tests.

QUESTIONS FOR DISCUSSION

1. Classification of obligate anaerobes: spore-forming clostridium, nonspore-forming microbes (*Bacteroides, Fusobacterium, Propionobacterium, Eubacterium, Peptococcus, Peptostreptococcus*).
2. Physiology of obligate anaerobic bacteria.
3. Methods of anaerobic culture isolation. Isolation of anaerobic bacteria in pure culture.
4. Anaerobic jar.
5. Biological investigation method, its application in the study of aetiology, pathogenesis, immunogenesis, diagnosis, therapy, and prevention of infectious diseases. Laboratory animals, animal lines.

PROCEDURE OF PRACTICAL WORK

Task 1. Study the demonstration.

Causative agent is rod-shaped, gram-positive. The bacteria are placed isolated and form the round terminal spore. The size of the spore is thicker than bacteria: the cell has the shape of drumstick. When smear is stained by Gram's method, the spore is colourless with violet contour. When smear is stained by Ziehl-Neelsen method, the spore as acid-stable structure is stained red, the body is stained blue.

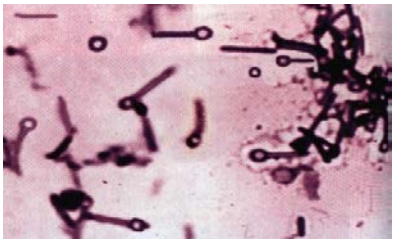


Figure 1.8.1 – *Clostridium tetani,* Gram staining

There are spore-forming anaerobes. *Clostridium perfringens* causes gas gangrene. *C. perfringens* is present throughout the environment. They are gram-positive spore-forming rods.

Figure 1.8.2 – *Clostridium perfringens,* Gram staining

Рис. 48. Мікроанаеростати

It is gram-positive streptococcus, nonspore-forming anaerobes.

Figure 1.8.3 – *Peptostreptococcus,* Gram staining

There are nonspore-forming anaerobes. They are members of the normal flora of the large intestine. They are gram-positive like branchy rods.

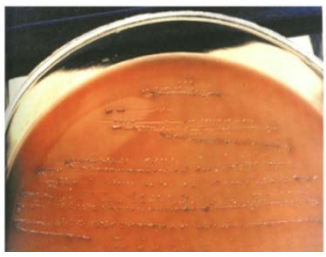
Figure 1.8.4 – *B.bifidum,* Gram staining

Anaerobic jar is an apparatus that can maintain constant temperature and it allows producing anaerobic conditions for cultivation. It is closed hermeticully. Vacuum pump pumps air out inside thecamera of the anaerobic jar.

Figure 1.8.5 – Anaerobic jar

Exsiccator may be used for anaerobes cultivation. The culture is placed inside exsiccator. The chemical method is used for the evolution of oxygen (the steam of the chemical matter that interacts with oxygen is placed on the bottom of the exsiccator).

Figure 1.8.6 – Exsiccator



Clostridium perfringens forms small round grey colony. This medium consists of glucose, blood, and agar. It is placed in Petri dish and used for isolation of the colony.

ис. 51. Рісг С.

Figure 1.8.6 – *Clostridium perfringens* on the blood-sugar Zeissler's agar

Skim milk is regenerated for oxygen absorption like Kitt-Tarozzi media. After 3–4 hours of the cultivation the like sponge clots rice. It consist of the blebs and transparent liquid. The tube on the left shows fermentation; the tube on the right is negative for stormy fermentation. Used for the identification of *Clostridium species*

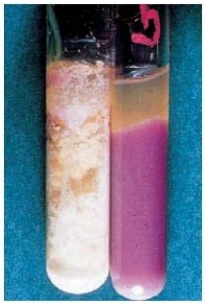


Figure 1.8.6 – Growth of *C. perfringens* on litmus milk

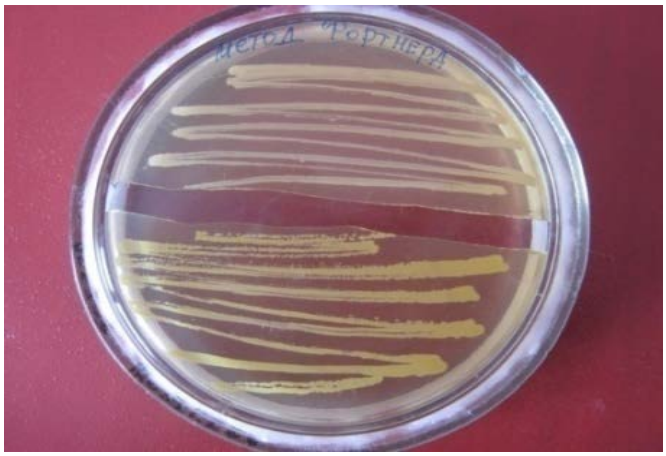
It is a nutrient broth with 0.5% glucose and pieces of minced liver and meat for oxygen absorption. Before inoculation the media is warmed thoroughly on the water bath during 10–15 minutes for the involution of oxygen (regeneration of the medim). After inoculation, the medium is covered by vaseline oil for prevention of interchange of gases with surroundings.

Figure 1.8.6 – Growth of *C. perfringens* on Kitt-Tarozzi medium

Wilson-Blerr medium is an iron-sulphate agar. It consists of nutrient medium with glucose, Na_2SO_3, $FeCl_2$. The medium is filling tubes like high column. The inoculation is making by prick of the nutrient medium. Anaerobic clostridia cause a blackening of the media in prick place because Na_2SO_3 reduce in Na_2S. It connects with $FeCl_2$ and makes black sediment.

The growth of *C. perfringens* on Wilson-Blerr medium is blackening in the deep of the Wilson-Blerr medium. Each dot represents an individual bacterial colony within the agar or on its surface. The surface, which is directly exposed to atmospheric oxygen, is aerobic. The oxygen content of the medium decreases with depth until the medium becomes anaerobic toward the bottom of the tube.

Growth of *C. perfringens* on Wilson-Blerr medium



Task 2. Study the principle of obligate anaerobic bacteria pure culture isolation and identification

Content film "Obligate anaerobic bacteria pure culture isolation and identification":

One of the main requirements in cultivating obligate anaerobic bacteria is removal of oxygen from the nutrient medium. The content of oxygen can be reduced by a great variety of methods: immersing of the surface of the nutrient medium with oile, introduction of microorganisms deep into a solid nutrient medium, the use of special anaerobic jars. Quantitative analysis of anaerobes, however, is best when performed in an anaerobic chamber such as an evacuated glove box filled with inert gas.

The Gaspak is now the method of choice for preparing anaerobic jars. The Gaspak is commercially available as a disposable envelope, containing chemicals which generate hydrogen and carbon dioxide on the addition of water. After ihe inoculated plates kept in the jar, the Gaspak envelope, with water added, is placed inside and the lid screwed tight hydrogen and carbon dioxide evolved and the presence of a cold calaryst in the envelope permits the combination of hydrogen and oxygen to produce an anaerobic environment The Gaspak is simple and effective, eliminating the need for drawing a vacuum and adding hydrogen. An indicator should be employed for verifying the anaerobic condition in the jars. Reduced methytene blue is generally used for this purpose. It remains colourless anaerobicaly but turns blue on exposure to oxygen.

Task 3. Study the principle of Brewer's anaerobic Petri dish.

Anaerobic bacteria may also be cultivated in special Petri dish without the use of complex and expensive incubators. One of the most convenient plate methods uses the Brewer's (named after John H. Brewer, an industrial bacteriologist, in 1942) anaerobic Petri dish, together with special anaerobic agar. Brewer's special cover fits on a normal Petri dish bottom in such a way that its circular ridge rests on the agar, thereby protecting most of the surface from the exposure to O_2. Brewer's anaerobic agar contains a high concentration of thioglycollic acid. The free sulfhydryl groups of thioglycollate reduce any O_2 present and create an anaerobic environment under the Brewer cover.

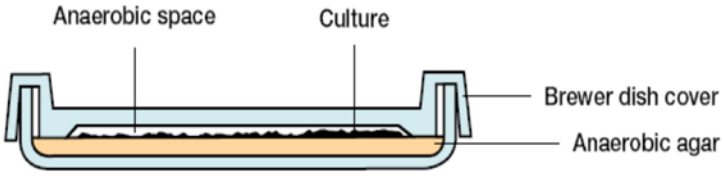
Task 4. Study the principle of Fortner's method and Vinyal-Veyon's method.

Fortner dish is a dish with Zeissler's agar and biological mechanical method of the anaerobic conditions making. The medium is divided into two parts (the furrow is made in the dish centre by scalpel). The aerobic and anaerobic bacteria are growing on the different parts of the medium.

Vinyal-Veyon's method is used for isolation of anaerobes colonies. It consists of the preparing the serial dilutions of the investigated materials in the fusion nutrient agar. The nutrient agar taken in the Paster pipettes with solder end. Thus, in that way, in the thickness of the medium anaerobic conditions are make. The pipette is placed into the thermostat with optimal temperature. The grown colony is obtained when the pipette is cut up or the end of the pipette is broken.

Obligate anaerobic bacteria.
Methods of anaerobic culture isolation. Biological method

Notion	Definition/explanation
Facultative anaerobes	Microorganisms that can live and grow with or without molecular oxygen
Obligate anaerobes	Microorganisms that can grow only in the complete absence of molecular oxygen; some are killed by oxygen



Metabolism	Obligate anaerobes may use fermentation or anaerobic respiration. Aerotolerant organisms are strictly fermentative. In the presence of oxygen, facultative anaerobes use aerobic respiration; without oxygen some of them ferment, some use anaerobic respiration
Examples of obligate	*Clostridium perfringens, C. tetani, C. histolyticum, C. novyi, C.*

anaerobic bacteria	*septicum, C. botulinum, Bacteroides fragilis, B. gracilis*
Anaerobic respiration	In the process of anaerobic respiration, carbohydrate can be metabolised by a process that uses oxidative phosphorylation via an electron transport chain, but instead of oxygen serving as the terminal electron acceptor (usually) inorganic molecule such as nitrate or sulphate is used. These processes are referred to, respectively, as dissimilatory nitrate or sulphate reduction. Obligate anaerobes carry out this process, as they are unable to use oxygen; in addition, other organisms may turn to this form of respiration if oxygen is unavailable (facultative anaerobes). Other examples of inorganic electron acceptors for anaerobic respiration include Fe^{3}+, CO_2 and Mn^{4}+. In certain circumstances, an organic molecule such as fumarate may be used

Table 1.8.1 continuation

Notion	Definition/explanation
	instead. Anaerobic respiration is not as productive as its aerobic counterpart in terms of ATP production, because electron acceptors such as nitrate or sulphate have less positive redox potentials than oxygen. Anaerobic respiration tends to occur in oxygen-depleted environments such as waterlogged soils. It must be stressed that anaerobic respiration is not the same as fermentation. The latter process does not involve the components of the electron transport chain (i.e., there is no oxidative phosphorylation), and much smaller amounts of energy are generated
Culturing anaerobes	Given that normal microbial culturing is undertaken in an aerobic environment, the culturing of anaerobes poses a problem. To overcome this, a number of techniques are employed by microbiologists. One way required the injection of the bacteria into a dicot. The dicot would then provide an environment without oxygen thus ensuring the survival of the anaerobes. The Gaspak system is an isolated container which achieves an anaerobic environment by the reaction of water with sodium borohydride and sodium bicarbonate tablets to produce hydrogen gas and carbon dioxide. Hydrogen then reacts with oxygen gas on a palladium catalyst to produce more water, thereby removing oxygen gas. The issue with the Gaspak method is that an adverse reaction can take place where the bacteria may die which is why a thioglycollate medium should be used. The thioglycollate supplies a medium mimicing that of a dicot thus providing not only an anaerobic environment but all the nutrients needed for the bacteria to thrive
Facultative anaerobic org anism	A facultative anaerobic organism is an organism, usually a bacterium, that makes ATP by aerobic respiration if oxygen is present but is also capable of switching to fermentation. In contrast, obligate

	anaerobes die in presence of oxygen
Examples of facultative anaerobic bacteria	The examples of facultative anaerobic bacteria are *Staphylococcus* (gram-positive), *Escherichia coli* (gram-negative), *Corynebacterium* (gram-positive), and *Listeria* (gram-positive)
Microaerophilic organisms	Microaerophilic organisms are a specific type of microorganism (especially bacteria) that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere (~20% concentration). Many microphiles are also capnophiles, as they require an elevated concentration of carbon dioxide. In the laboratory they can be easily cultivated in a candle jar, a container into which a lit candle is introduced before the airtight lid impression. The flame burns until extinguished by oxygen deprivation, creating a carbon dioxide-rich, oxygen-poor atmosphere
Some examples of microaerophilic organisms	*Borrelia burgdorferi*, a species of spirochaete bacteria that causes Lyme disease in humans. *Helicobacter pylori*, a species of proteobacteria that has been linked to peptic ulcers and some types of gastritis. Some don't consider it a true obligate microaerophile. *Campylobacter* has been described as microaerophilic. *Streptococcus intermedius* has also been described as microaerophilic
Aerotolerant anaerobes	Aerotolerant anaerobes are microorganisms that cannot use O_2 but are not harmed by it either (*Clostridium histolyticum*)

Notion	**Definition/explanation**
Methods of anaerobic culture isolation	Physical method → cultivated the anaerobic bacteria's in agar deep or in fluid media, but on surface presence oil, Vinyal-Veyon's method. Chemical method → used in media substance for absorption O_2. Biological method → Fortner method (subcultured to aerobic and anaerobic)
Isolation and identification of pure culture of anaerobic bacteria	**First day.** Inoculate the studied material into Kitt-Tarozzi medium (nutrient medium): concentrated nutrient broth or Hottinger's broth, glucose, 0.15 % agar (pH 7.2–7.4). To adsorb oxygen, place pieces of boiled liver or minced meat to form a 1–1.5 cm layer and pieces of cotton wool on the bottom of the test tube and pour in 6–7 min of the medium. Prior to inoculation place the medium into boiling water for 10-20 min in order to remove air oxygen contained in it and then let it cool. Upon isolation of spore forms of anaerobes the inoculated culture is reheated at 80 °C for 20–30 min to kill nonspore-forming bacteria. The cultures are immersed with petrolatum and placed into an

	incubator. Apart from Kitt-Tarozzi medium, liquid media containing 0.5–1 % glucose and pieces of animal organs, casein-acid and casein-mycotic hydrolysates can also be employed. **Second day.** Take note of changes in the enrichment medium, namely, the appearance of either opacity or opacity with gas formation. Take broth culture with a' Pasteur pipette and transfer it through a layer of petrolatum onto the bottom of the test tube. Prepare smears on a glass slide in the usual manner, then flame fix and Gram-stain them. During microscopic examination record the presence of Gram-positive rod forms (with or without spores). Streak the culture from the enrichment medium onto solid nutrient media. Isolated colonies are prepared by two methods. 1. Prepare three plates with blood-sugar agar. To do it, melt and cool to 45 °C 100 ml of 2 % agar on Hottinger's broth, then add 10–15 ml of deftbrinated sheep or rabbit blood and 10 ml of 20 % sterile glucose. Take a drop of the medium with microorganisms into the first plate and spread it along the surface, using a glass spatula. Use the same spatula to streak the culture onto the second and then third plates and place them into an anaerobic jar or other similar devices at 37 °C for 24–48 hrs (Zoisslcr's method). 2. Anaerobic microorganisms are grown deep in a solid nutrient medium (Veinherg's method of sequential dilutions). The culture from the medium is taken with a Pasteur pipette with a thin tip and transferred consecutively into the 1st, 2nd, and 3rd test tubes with 10 ml of isotonic sodium chloride solution. Continue to dilute transferring the material into the 4th, 5th. and 6th thin-walled test tubes (0.8 cm in diameter and 18 cm in height) with melted and cooled to 50 °C nutrient agar or Wilson-Blair medium (to 100 ml of melted nutrient agar with 1 % glucose add 10 ml of 20 % sodium sulphite solution and 1 ml of 8 % ferric chloride). Alter agar has solidified, place the inoculated culture into an incubator. **Third day**. Study the isolated colonies formed in third plate and make smears from the most typical ones. The remainder is inoculated into Kitt-Tarozzi medium. The colonies in the test tubes are removed

Notion	Definition/explanation
	by means of a sterile Pasteur pipette or the agar column may be pushed out of the tube by steam generated upon warming the bottom of the test tube. Some portion of the colony is used to prepare smears, while its remainder is inoculated into Kitt-Tarozzi medium to enrich pure culture to be later identified by its

	morphological, cultural, biochemical, toxicogenic, antigenic, and other properties (on the forth-fifth day). The Vinyal-Veyon's method is used for mechanical protection from oxygen. The seeding are made into tube with melting and cooling (at 42 $^{\circ}$C) agar media
Biological method	For this method using various laboratory animals. Most often used in case of impossibility of cultivation of bacteria on nutrient media
Experimental or biological method for diagnostics of antrax	The anthrax bacillus can often be isolated from contaminated tissues by applying them over the shaven skin of a guinea pig. The animal dies in 24–72 h, showing a local, gelatinous, haemorrhagic oedema at the site of inoculation, extensive subcutaneous congestion and characteristically, an enlarged, dark red, friable spleen. The blood is dark red and coagulates less firmly than normally. The bacilli are found in large numbers in the local lesion, heart blood and spleen (more than 10^8 bacilli/ml)

BACTERIAL GENETICS. BACTERIOPHAGES

Theme topicality. Genetic mechanisms have opened new perspectives in the diagnosis of infectious diseases. In addition, it is the reason of the microorganisms resistance to antibiotics. Polymerase chain reaction, which is now widely used for diagnostics is also based on genetic principles.

Primary objective: to be able to conduct and evaluate the experiments on the genetic recombination; to learn the principles of the phagotyping and the use of bacteriophage in medicine.

QUESTIONS FOR DISCUSSION

1. Genetics of microorganisms.
2. Genotypic and phenotypic variations.
3. Transmission of genetic material.
4. Plasmids: definition, classification, function.
5. Bacteriophages. Structure of bacteriophages. The life cycle of bacteriophages.
6. Practical using of bacteriophages.
7. Polymerase chain reaction (PCR).

PROCEDURE OF PRACTICAL WORK

Task 1. Perform and read conjugation reaction.

For conjugation reaction 2 cultures are used:

1. *E. coli* F+ Pro+, Ura+, His+, StrS. This culture has fertility-factor, plasmid and is able to produse proline, uracile, histidin, but it is sensitive to streptomycin.

2. *E.coli* F-, Pro-, Ura-, His-, StrR, it is resistant to streptomycin.
Basal medium is used for this experiment. In this medium amino acid is absent, but streptomycin is present.

Principle of conjugation reaction

+

Figure 1.9.1 – Principle of conjugation reaction

Task 2. Perform and read transformation reaction.

In this expirement of transformation one should use:
1. Recipient culture is *S. aureus*, this culture is sensitive to streptomycin.
2. DNA, with donor culture, in this DNA the genes resistant to streptomycin are present.
3. Selective medium, with streptomycin.

Figure 1.9.2 – Principle of transformation reaction

Task 3. Perform and read demonstration on plural resistance of bacterial culture of *Staphylococcus aureus* to the antibiotics.

After measuring a zone of the absence of growth round a paper disk, write down the result of determination of sensitivity to antibiotics in the report (what preparation can be recommended for treatment of the patient).

Task 4. Study the bacteriophages for treatment, prophylaxis, and diagnosis purposes.

Bacteriophages for treatment, prophylaxis, and diagnosis purposes

Notion	Definition/explanation
Polyvalent shigellosis bacteriophage (liquid)	It is diagnostic preparation which contains the sterile filtrate of bacteriophages, that lyses *S. flexneri* and *S. sonnei*. It is used for phagotyping the selected pure culture of shigellae (to confirm isolated pure culture of shigellae to *Shigella* genus)
Polyvalent shigellosis bacteriophage (in tablet)	It is treatment and prophylactic preparation contains the sterile filtrate of bacteriophages, that lysis *S. flexneri of serotype 1–6* and *S. sonnei*. Produced as tablets with acid-resisting coverage. It is used for the emergency prophylaxis of shigellosis and treatment of acute shigellosis
Salmonellosis	It is preparation, containing sterile filtrate of bacteriophages lysing

Notion	Definition/explanation
polyvalent A,B,C,D,E bacteriophage (tabletted)	*S. paratyphi A, S. schottmuelleri, S.typhimurium, S. heidelberg, S. newport, S. infantis, S. choleraesuis, S. oranienburg, S. dublin, S.enteritidis, S. gallinarum, S. anatum, S. newlands.* It is produced in tablets with acid resistance coverage. It is used for salmonella infection urgent prophylaxis and treatment

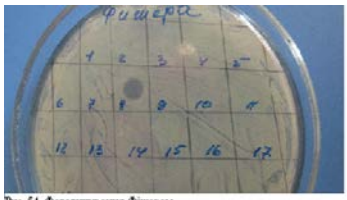


Рис. 54. Фаготипування Фішером

Cholera bacteriophage El Tor (liquid)	It is diagnostic preparation which contains the sterile filtrate of bacteriophages, that lysis *V. cholera biotype El Tor.* It is used for phagotyping the selected pure culture of cholera (for confirm isolated pure culture of cholerae to *V. cholera biotype El Tor*)

Task 5. Perform the identification of the bacterial cultures by means of phagotyping.

Principle of the phagotyping method

It is used for the rapid diagnosis of plague and some other infections. Native material to be tested or the culture isolated from this material is streaked on Petri dish. Then in the upper sector of the dish the specific phage is poured, homologous to the culture to found or to the species to which this culture hypothetically is related. Then the dish is placed vertically so that the drop slips by gravity downwards, forming on the course a path.

After this procedure the dish is put to thermostat at 37 °C.

If the material to be tested contains microorganisms homologous to the phage or the isolated culture is homologous to the phage, then at the way of the path the microbial growth is absent, while at the rest of the nutrient media it is present.

Bacteriophages are available that lyse all members of the bacterial genus (e.g., genus specific bacteriophage for *Salmonella),* all members of a species (e.g., specific bacteriophage for *B. anthracis),* and all members of a biotype or subspecies (e.g., Mukerjee's phage IV which lyses all strains of classical *V. cholerae* but not *V. cholerae* biotype El Tor).

Identification of the cultures is to be tested by specific bacteriophages method of path.

Bacterial genetics. Bacteriophages

Notion	Definition/explanation
Genetics, genome and chromosomes	Genetics is the science of heredity. It includes the study of what genes are, how they carry information, how they are replicated and passed to subsequent generations of cells or passed between organisms, and how the expression of their information within an organism determines the particular characteristics of that organism. The genetic information in the cell is called genome. Cell’s genome includes its chromosomes and plasmids. Chromosomes are structures containing DNA that physically carry hereditary information; the chromosomes contain the genes

Genotype and phenotype	The genotype of an organism is its genetic makeup, the information that codes for all the particular characteristics of the organism. The genotype represents potential properties, but not the properties themselves. Phenotype refers to actual, expressed properties, such as the organism's ability to perform a particular chemical reaction. Phenotype, then, is the manifestation of genotype
Transcription	Transcription is the syn thesis of a complementary strand of RNA from a DNA template
Transformation	Naked DNA fragments from one bacterium, released during cell lysis,

Table 1.9.2 continuation

Notion	Definition/explanation
	bind to the cell wall of another bacterium. The recipient bacterium must be competent, which means that it has structures on its cell wall that can bind the DNA and take it up intracellularly. Recipient competent bacteria are usually of the same species as the donor. The DNA that has been brought in can then incorporate itself into the recipient's genome if there is enough homology between strands (another reason why this transfer can only occur between closely related bacteria)
Transduction	Transduction occurs when a virus that infects bacteria, called a bacteriophage, carries a piece of bacterial DNA from one bacterium to another.
Type of transduction	Just as there are two types of phages, there are two types of transduction. Virulent phages are involved in generalized transduction and temperate phages in specialized transduction.
Bacterial Viruses	Virulent bacteriophages, lytic life cycle (e.g.,T4): 1.Adsorption of phage to host. 2.Penetration of virus genetic material. 3.Replication, synthesis of virus DNA and proteins. 4.Cell lysis and release of phage particles. Temperate bacteriophages, lysogenic life cycle (e.g., λ): 1.Adsorption of phage to host. 2.Penetration of virus genetic material. 3.Integration of virus into host chromosome, prophage. 4.Replication along with host chromosome, maintained as a prophage
Two types of phages	There are two types of phages: virulent phages and temperate phages. Virulent phages infect the bacteria, reproduce, and then lyse and kill the bacteria. On the other hand, temperate phages have a good temperament and do not immediately lyse the bacteria they infect. The temperate phage undergoes adsorption and penetration like the virulent phage but then, rather than undergoing transcription, its DNA becomes incorporated into the bacterial chromosome. The DNA then waits for a command to activate

Types of phage reproduction in bacterial cell	Productive type: bacteriophages lyse their host bacterial cells after penetration and reproductive cycle. Abortive type: after penetration the new phage particles will not forming and bacteria live. Integrative type: phage genome is integrated in bacterial chromosome and bacterial host cell is live
Classification of bacteriophages	Polyvalent – destroy kindred bacteria, for example, polyvalent salmonella bacteriophages destroy all *Salmonella spp*. (*S. enteritidis*, *S. typhimurium*). Monovalent – destroy one species of bacteria, for example, typhoid fever Vi- bacteriophages – destroy only typhoid fever agent. Typospecific – destroy one type of bacteria species
The importance of the bacteriophages	**Biological investigation** Bacteriophages are often used as models in studies of fundamental biological processes: DNA replication, gene expression, gene regulation, viral morphogenesis, studies of the details and function of supramolecular structures. **Genetic engineering** Vectors for gene cloning, adjuvants in sequencing **Therapy and prevention**

Table 1.9.2 continuation

Notion	Definition/explanation
	An older concept now receiving increased attention. Administration of suitable phage mixtures in therapy and prevention of gastrointestinal infections. In animal husbandry, a number of phages that attack only EHEC (enterohaemorrhagic *E. coli*) are used against EHEC infections. **Epidemiology** Bacterial typing. Strains of a bacterial species are classified in phagovars (syn. lysotypes) based on their sensitivity to typing bacteriophages. Recognition of the bacterial strain responsible for an epidemic, making it possible to follow up the chain of infection and identify the infection sources. This typing method has been established for *Salmonella typhi*, *Salmonella paratyphi B*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and other bacteria, although it is now increasingly being replaced by new molecular methods, in particular DNA typing
Prophage	The integrated temperate phage genome is called a prophage. Bacteria that have a prophage integrated into their chromosome are called lysogenic because at some time the repressed prophage can become activated. Once activated, the prophage initiates the production of new phages, beginning a cycle that ends with bacterial cell lysis. So

	temperate phages, although of good temperament, are like little genetic time bombs
Conjugation	Conjugation is bacterial sex at its best: hot and heavy. In conjugation DNA is transferred directly by cell-to-cell contact, resulting in an extremely efficient exchange of genetic information. The exchange can occur between unrelated bacteria and is the major mechanism for transfer of antibiotic resistance
Mutation	Any alteration made to the DNA sequence of an organism is called a mutation. This may or may not have an effect on the phenotype (physically manifested properties) of the organism. 1. Morphological mutations change microorganisms appearance, colony or cellular morphology. 2. Lethal mutations-result in death of microorganism. 3. Conditional mutations are expressed under certain environmental conditions, e.g., not expressed at a low temperature (permissive temp), but expressed at high temperature (non-permissive temp). 4. Biochemical mutations alter a biosynthetic pathway and the organisms ability to grow on minimal media. 5. Resistant mutations – mutant that is now resistant to an antibiotic or virus
Spontaneous mutation	Spontaneous mutation occurs randomly, may result from errors during DNA replication
Group of plasmids includes	The group of plasmids includes : 1. the genome of the temperate phage; 2. the fertility factor (F- factor); 3. the factor of transmission of multiple resistance to drugs (R-factor); 4. other the haemolytic, enterotoxigenic, urease factors, the factor of bacteriocinogenesis, etc.
Plasmids	Plasmids are small ds DNA molecules, usually circular that can exisit independently of the host chromosome. They have their own replication origin so can replicate automonously (replicon) and

Table 1.9.2 continuation

Notion	**Definition/explanation**
	have relatively few genes (<30) that are not essential to the host
Conjugative plasmids	Conjugative plasmids have genes for pili and can transfer copies of themselves to other bacteria during conjugation.
Fertility factor or F factor	Fertility factor or F factor – these plasmids can also intergrate into the host chromosome or be maintained as an episome (independent of chromosome)
R factor	Conjugative plasmids which have genes that code for antibiotic

	resistance for the bacteria harbouring them. These do not integrate into the host chromosome
Col Plasmids	Harbour bacteriocins which are proteins that destroy other bacteria (e.g., cloacins kill *Enterobacter species*)
Virulent plasmids	Virulent plasmids – have genes which make bacteria more pathogenic because the bacteria is better able to resist host defences or produce toxins/invasions
Antibiotic resistance	Resistance to antibiotics can be acquired as genes are transferred between bacteria (e.g., R plasmids or transposons). Many infections are treated with antibiotics and the increasing number of drug resistant pathogens is a serious public problem. Antimicrobial agents kill or stop growth of various pathogens: 1. Inhibit cell wall synthesis. 2. Affect protein or nucleic acid synthesis. 3. Disrupt membrane structure and function. 4. Block metabolic pathways. Mechanisms by which bacteria resist antibiotic treatment: 1. Inactivation of antibiotic through chemical modification. 2. Change in target of antibiotic to inhibit action
Analysis of DNA	1. Gel electrophoresis separates DNA fragments based on size. 2. Nucleic acid hybridization and probes – probes base pair with complementary sequences; used to detect specific sequences. 3. DNA sequencing – reading the sequence of nucleotides in the DNA stretch. 4. Polymerase chain reaction – way to amplify DNA

Abreviation

ABS – antigen-binding site
BGEC – Bacteria Group of *Escherichia coli*
CFT – complement fixation test
CNS – central nervous system
DLM – doses letalis minima
DTH – delayed type of the hypersensitivity
ELISA – enzyme-linked immunosorbent assay
EMB – Eosin methylen blue
EPEC – enteropathogenic *E. coli*
EIEC – enteroinvasive *E. coli*
ETEC – enterotoxigenic *E. coli*
EHEC – enterohaemorrhagic *E. coli*
EYA – egg yolk agar

FA – fagocytic activity
FI – fagocytic index
FACS – fluorescence-activated cell sorter
GAS – group A streptococci
IFT – immunofluorescent test
IU – international units
MBT – *Mycobacterium tuberculosis*
MIC – minimal inhibition concentration
MHC – major histocompatibility complex
MPA – meat pepton agar
MRSA – methicillin-resistant Staphylococcus aureus
NBT – nitrat blue tetrasolium
NT – neutralization test
PHAT – indirect (passive) hemagglutination test
PMNL – polimorphonuclear leucocyte
RPR – rapid plasma reagin
RIA – radioimmunoassay
STSS – Streptococcal toxic shock syndrome
TCBS – thiosulfate-citrate-bile salts-sucrose
VDRL – venereal disease research laboratory

QUIZZES

1. Choose the nutrient medium for obtaining the separated colonies:
A. Meat-peptone broth
B. Sugar broth
*C. Meat-peptone agar
D. Kitt-Tarozzi medium
E. Alkaline peptone water

2. We can obtain pure culture of anaerobic microorganisms with the help of:
A. Fortner's method

B. Shukevich's method
C. Paster's method
*D. Weinberg’s method
E. Loeffler's method

3. What is the value of a hanging-drop preparation?
A. For study sensitivity to antibiotics
B. For examination of biochemical properties
C. For study morphology of bacteria
*D. For study motility of bacteria
E. For study cultural properties of bacteria

4. Choose obligate anaerobes among these bacteria:
A Neisseria gonorrhoeae, Streptococcus pneumoniae
B. Mycobacterium tuberculosis
*C. Clostridium tetani, Clostridium botulini
D. Bacillus anthracis, Brucella melitensis
E. Shigella dysenteriae, Salmonella typhi

6. The second stage of obtaining the pure culture of aerobes is:
A. Inoculating of a mix (material) on a solid nutrient medium
B. Check of cleanliness of the pure culture and its identification
C. Biochemical and serological identification
*D. Studying of isolated colonies and inoculating on the slant agar
E. Microscopic examination of a material

7. The third stage of obtaining the pure culture of aerobes is:
A. Check of cleanliness of the pure culture and its identification
B. Inoculating of a mix (material) on a solid nutrient medium
*C. Biochemical and serological identification
D. Studying of isolated colonies and inoculating on the slant agar
E. Microscopic examination of a material

8. We can study proteolytic properties of bacteria by detection in tubes of:
A. Carbonic acids and waters
B. Glucose and lactose
C. Carbonic acids and nitrogen
D. Manitolum and methanol
*E. Indol and hydrogen sulphite

9. What microorganism is a sanitary – indicative in studying of quality of running water?
A. Staphylococcus aureus

B. Corynebacterium diphtheriae
C. Streptococcus pneumoniae
*D. Escherichia coli
E. Vibrio cholerae

10. To check up the work of autoclave a laboratory assistant used a chemical substance melting in temperature 119^0 C. What is this substance?
A. Nitrogen
B. Potassium
C. Benzyl
D. Silver
*E. Sulfur

11. While studying inoculations of air taken from chemists a bacteriologist found a sanitary – indicative microorganism. What is this microorganism?
A. Escherichia coli
B. Vibrio cholerae
C. Streptococcus pneumoniae
***D. Staphylococcus aureus**
E. Mycobacterium tuberculosis

12. A laboratory assistant sterilized simple nutritious media and physiological solution in an autoclave. What regime of work was used in the autoclave?
*A. 120 degree C (1atm.)
B. 115 degree C (0,5atm.)
C. 100 degree C (0atm.)
D. 134 degree C (2atm.)
E. 148 degree C (3atm.)

13. In inoculation of faeces for nutritious medium both the red colonies with metallic luster and colorless colonies developed. How do we call this nutritious medium?
A. Blood agar
B. Chocolate agar
C. Kitt - Tarrozzi medium
*D. Endo medium
E. Hiss medium

14. To isolate an agent of anaerobic infection a bacteriologist made an inoculation of studied material into liquid nutritious medium covered by oil. How do we call this medium?
A. Endo medium
B. Chocolate agar

C. Hiss medium
D. Blood agar
*E. Kitt – Tarrozzi medium

15. The answer from laboratory indicates the minimal therapeutic dose of antibiotics which the agent is sensitive to. In what method was this dose determined?
A. Membrane filter method
B. Sedimentations method
C. Serological method
*D. Dilution test
E. Diffusion test

16. To determine the agents sensitivity to antibiotics the method was used in which the small filtrating paper disks soaked with antibiotics are brought on the medium inoculated with staphylococci culture. How do we call this method?
A. Sedimentations method
B. Serological method
*C. Diffusion test
D. Membrane filter method
E. Dilution test

17. At the chemists a medical preparation was sterilized by the method of tindalization. What apparatus was used?
A. Hot air oven
B. Membrane filter
C. Autoclave
*D. Water bath
E. Steamer

18. To characterize the qualities of medicinal preparation, a chemical – therapeutical index was introduced. The index was proposed by:
A. Lister
B. Neisser
C. Leeuwenhoek
D. Robert Hook
*E. Ehrlich

19. To isolate an agent, a bacteriologist inoculated by stroke a studied material on dense nutrious medium in a Petri dish divided into four sectors. What method of isolation of pure culture was used?
A. Biological
B. Bacteriological

*C. Mechanical
D. Allergic
E. Serological

20. A bacteriologist reveled in a strain of colon bacillus the ability to work out the colicines. What is this quality provided by?
A. Flagella
B. Mesosomes
C. Spores
D. Ribosomes
*E. Plasmids

21. Staphylococcus is isolated from a discharge of middle ear. A bacteriologist inoculated an isolated culture into a test – tube with citrate rabbit plasma. What enzyme of aggression did he want to reveal?
*A. Coagulase
B. Hemolysin
C. Leucocidin
D. Fibrinolysin
E. Hyaluronidase

22. Pathogenic staphylococcus was isolated from a suppurative wound of a patient, its sensitivity to antibiotics was determined. Penicillin caused a zone of growth retention – 8mm; oxacillin – 9mm; ampicillin – 10mm; gentamicin – 22mm; lincomycin – 11mm. What antibiotic should be chosen for treatment of the patient?
A. Penicillin
***B. Gentamicin**
C. Ampicillin
D. Lincomycin
E. Oxacillin

23. While determining the sensitivity staphylococcus to antibiotics, the following results were obtained: diameter of zone of growth retention of penicillin – 7mm; oxacillin – 8mm; ampicillin – 26mm; gentamicin – 22mm; lincomycin – 15mm. What method of investigation was used?
A. Bacteriological
B. Serological
*C. Diffusion test
D. Chemical test
E. Dilution test

24. Nutrious media containing substances destroying in temperature more than 100 degree C (urea, carbohydrates, proteins) should be sterilized for

bacteriological examination. What method of sterilization can be used in this case?
A. Sterilization by filtration
B. Ultraviolet radiations
*C. Tindalization
D. Autoclavation
E. Infra red radiation

25. To obtain exotoxin, a toxigenic microorganism is inoculated into liquid nutrious medium. After toxins accumulation in the medium, microbe cells are removed from it. What device is used in this case?
A. Autoclave
B. Boiling
*C. Membrane filter
D. Hot air oven
E. Steamer

26. To confirm clinical diagnosis of an infectious disease, a laboratory study is carried out. What method of isolation of pure culture of microorganisms is used?
A. Serological
B. Biological
C. Bacterioscopical
*D. Bacteriological
E. Allergic

27. Corresponding nutrious media are used in bacteriological laboratories to isolate a pure culture and identify the isolated microorganism. What medium is used to isolate and identify staphylococcus?
A. Endo medium
B. Hiss medium
*C. Blood agar
D. Chocolate medium
E. MPA

28. At present the description of ten kinds of staphylococci is represented. They are revealed in people, domestic and animals. But only one kind is of great importance in the infectious pathology of man. How do we call this kind?
A. Staphylococcus epidermidis
B. Staphylococcus saprophyticus
C. Staphylococcus albus
*D. Staphylococcus aureus
E. Staphylococcus citreus

29. When studying the microflora of air in chemists, a pure culture of a microorganism was isolated. The microorganism grows and develops if there is not less than 20 per cent oxygen in atmosphere. What group does an agent belong to according to the type of breathing?
A. Microaerophiles
B. Facultative anaerobes
C. Obligate anaerobes
*D. Obligate aerobes
E. Aerotolerant microorganisms

30. The consignment of battles with glucose solution for parenteral use in prepared in a chemists. What way of sterilization is necessary to use?
A. Sterilization by filtration
*B. Autoclavation
C. Tindalization
D. Hot air over
E. Ultraviolet radiation

31. The formation of acquired stability to antibiotics occurs not only by the way of selection of spontaneous antibiotic stable mutants of the bacteria, but also due to spreadening among microorganisms particular factors which are called:
A. F - pili
*B. R - plasmid
C. A - protein
D. Spore
E. Cell wall

32. Sanitary – indicative microorganisms are chosen for characteristics of bacterial contamination of soil by man and animal. What microorganism present in soil is an index of long fecal contamination?
A. Staphylococcus
B. Enterococcus
*C. Clostridia
D. Neisseria
E. Mycobacteria

33. Chemical substances destroying microorganisms in environment are called disinfecting. What concentration of ethyl alcohol is most effective to manifest antimicrobic effect?
A. 100 degree
*B. 70 degree
C. 80 degree

D. 40 degree
E. 50 degree

34. Which of the following is an alkylation agent used as a disinfectant?
Merbromin
Iodouridine
Silver nitrate
Phenol
*E.Glutaraldehyde

35. What antimicrobial agent displays specificity for anaerobic microorganisms?
*A. Metronidazole
B. Polymyxin
C.Vancomycin
D.Gentamicin
E. Oxacillin

36. What is the single most frequent etiologic agent of ascending urinary tract infection?
A. Klebsiella pneumoniae
B. Serratia marcescens
C. Citrobacter freundii
D. Enterobacter cloacae
E. **Escherichia coli*

37. Which bacteria are most numerous in the human gastrointestinal tract?
A. Escherichia species
B. **Bacteroides species*
C. Pseudomonas species
D. Enterobacter species
E. Proteus species

38. Staphylococcus aureus is isolated from a pus. A bacteriologist plated an isolated culture on blood agar. What enzyme of aggression did he want to reveal?
A. Fibrinolysin
B. Hyaluronidase
C. **Hemolysin*
D. Leucocidin
E. Endotoxin

39. Escherichia coli is isolated from an intestinal discharge. A bacteriologist reveled in a strain the ability to fermentative the lactose. What nutritious medium he was used?

A. Hiss medium
B. **Endo medium*
C. MPW
D. Blood agar
E. MPA

40. For the identification of the microorganisms it is necessary to determine their proteolytic activity. What nutritious medium can be used for it?

A. **Meat peptone water*
B. Meat peptone agar
C. Blood medium
D. Kitt – Tarrozzi medium
E. Endo medium

41. The operation theatre was sterilized. What physical method of sterilization can be used for it?

A. Autoclavation
B. Tindalization
C. **Ultraviolet radiation*
D. Filtration
E. Hot air oven

Recommended reading list

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Informational resourses:

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