AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lecture 10.

Basics of clinical microbiology. Healthcare-associated infections. Infections of the respiratory tract, gastrointestinal tract, urogenital tract, central nervous system, wound and septic infections

FACULTY: ***General Medicine***
SUBJECT: ***Medical microbiology - 2***

Lecture plan:

1. The concept of clinical microbiology. Information on healthcare-associated infections. Infection Control.

2. Upper and lower respiratory tract, brief anatomical and physiological information

- Normal microflora of the upper respiratory tract, inflammatory diseases and their causes, rules for taking pathological material, principles of microbiological diagnosis

- Inflammatory diseases of the lower respiratory tract, their types, causative agents, principles of microbiological diagnosis.

3. Gastrointestinal tract, brief anatomical and physiological information

- Concepts of normal microflora of the gastrointestinal tract, dysbiosis and dysbacteriosis

- Inflammatory diseases of the gastrointestinal tract and their causes, principles of microbiological diagnosis

- Criteria and microbiological diagnosis of dysbiosis

4. Central nervous system, brief anatomical and physiological information

- Inflammatory diseases of the brain and meninges, meningitis, encephalitis, causative microorganisms, principles of microbiological diagnosis.

5. Urinary tract and genitals, brief anatomical and physiological information

- Normal microflora of the genitourinary tract, inflammatory diseases and their causative agents.

- Microbiological examination of urine. Bacteriuria, its determination and evaluation.

- Sexually transmitted diseases.

- Inflammatory diseases of female genital organs, microbiological examination methods.

- Understanding of TORCH infections. Transplacental diseases and their diagnosis.

- Inflammatory diseases of male genital organs, microbiological examination methods.

6. Inflammatory diseases of the skin. Complicated infections during skin lesions.

- Wound infections, microbiological diagnosis.

7. Sepsis, causative agents of septic infections, principles of microbiological diagnosis.

Clinical microbiology

- Clinical microbiology studies the microbiology of diseases of organs and systems of the human body, the principles of their microbiological diagnosis.

The microbiology and diagnostics principles of the nosocomial infections

Nosocomial infections

- Nosocomial infections (Greek, "nosocomion" - hospital) infections usually develop 48 hours after admission to the hospital and sources of infection may be carriers, contaminated medical instruments and equipment, medical personnel, or people visiting patients.
- Nosocomial infections are common in hospitals and, combined with the underlying disease, exacerbate its course.
- They can be caused by both pathogenic and opportunistic microorganisms.

The causative agents of nosocomial infections

- The causative agents of nosocomial infections are often opportunistic microorganisms.
- The range of pathogens of nosocomial infections is very wide, they are caused by various viruses, bacteria, fungi and protozoa.
- Among the causative bacteria are staphylococci, enterococci, pneumococci, enterobacteria, P. aeruginosa and other non-fermenting bacteria, anaerobes.
- Recently, there has been an increase in nosocomial infections caused by respiratory viruses and Candida fungi.

Characteristics of nosocomial infections

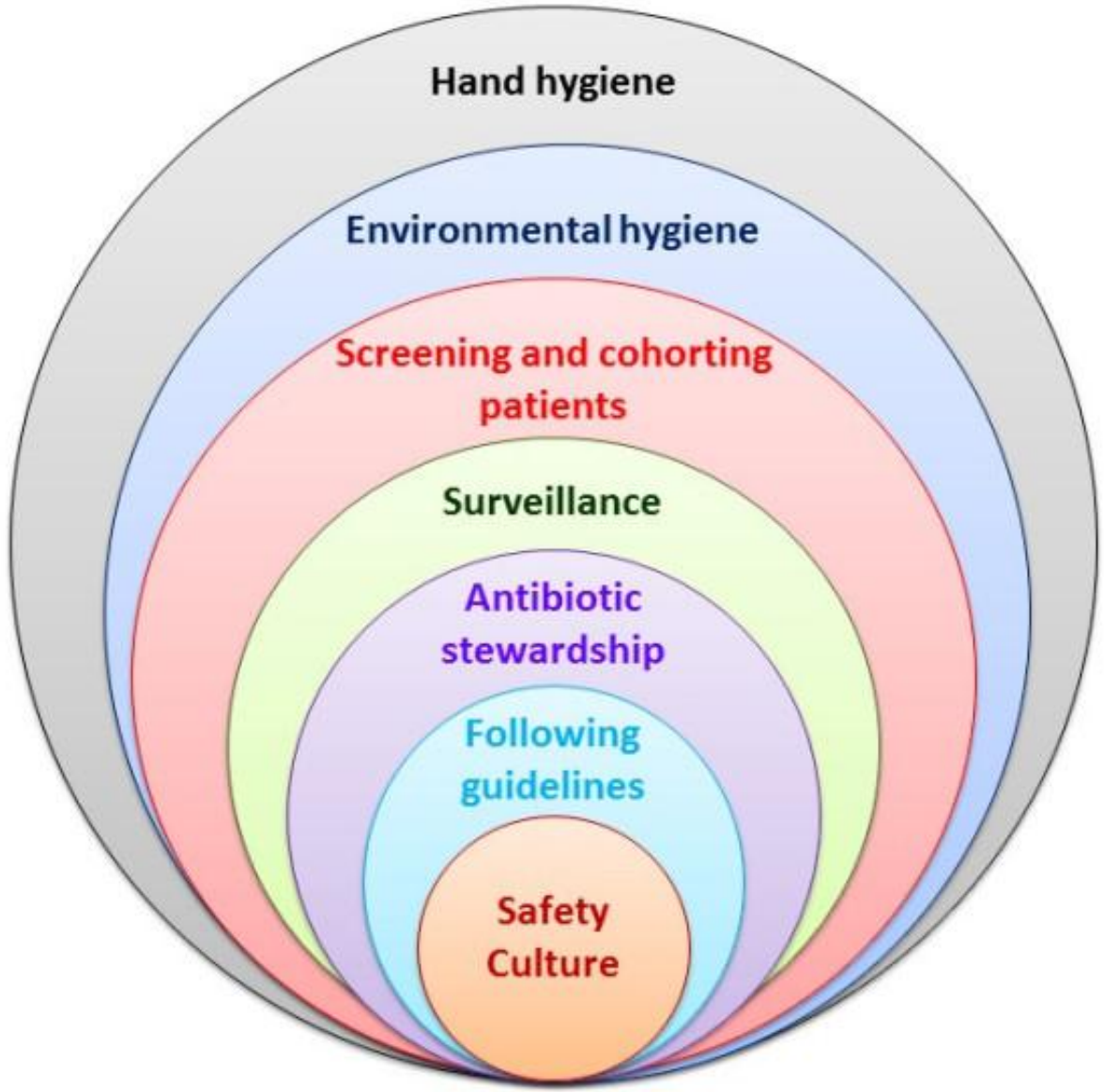
Opportunistic microorganisms that cause nosocomial infections differ in the following features:

- The causative agents of nosocomial infections are more resistant to antibacterial drugs, antiseptics, disinfectants, physical agents, bacteriophages and bacteriocins.
- Bacteria obtaining during nosocomial infections as a rule have higher virulence.
- Since opportunistic microorganisms do not have organ tropism, they can cause diseases in any organ and tissue of the body
- Heterogeneity (antigen variability, etc.) in the population of nosocomial infections is higher than in other microorganisms.

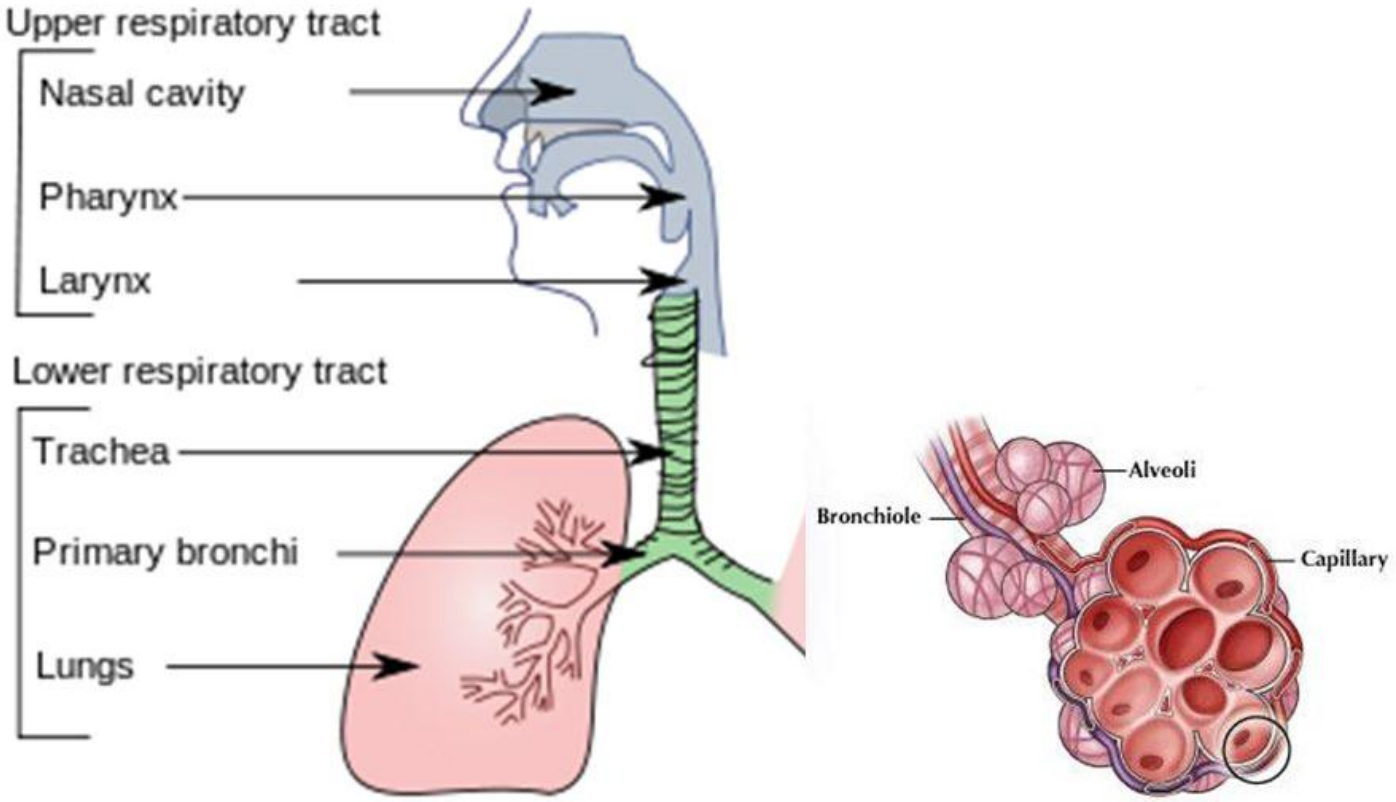
Principles of microbiological diagnosis of nosocomial infections

- Materials for investigation are selected depending on the location and nature of the disease.
- **The microscopic method** allows to make approximate conclusions about the nature of the causative agents and to determine the direction of cultural methods. Depending on the nature of the test material, the smears are then microscopic after staining with appropriate methods.
- **The culture method** is the main method of microbiological diagnosis in nosocomial infections. It is important to determine the sensitivity of the culture to antibiotics and other antimicrobial chemical therapeutic drugs.

7 strategies to prevent healthcare-associated infections



Principles of microbiology and diagnosis of respiratory infections



Upper respiratory tract (anatomy and normal microflora)

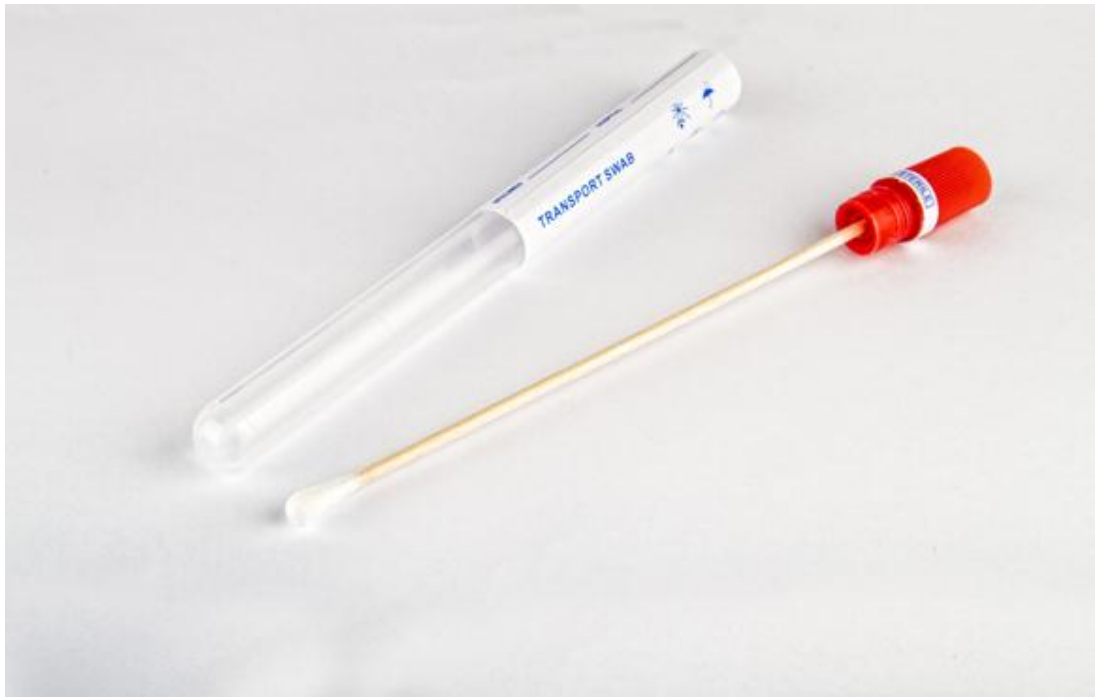
- Inflammatory diseases of the upper respiratory tract - pharynx and larynx are often caused by pathogenic microorganisms.
- Nasal mucosa encountered non-patogenic corynebacteria, coagulasa-negative staphylococci, alpha-hemolytic streptococci, Neisseria, sometimes potential pathogen bacterias such as S.aureus, beta-hemolytic streptococci, S.pneumoniae, E.coli, Proteus species.

Microbiology of upper respiratory tract infections

- **Rhinitis and sinusitis** (haimoritis, ethmoiditis, etc.) - adenoviruses, rhinoviruses, coronaviruses, etc.
- **Pharyngitis or angina** (refers to inflammation of the pharynx, soft palate and pharynx) and tonsillitis (inflammation of the tonsils)
- **Catarrhal pharyngitis** - ortho- and paramyxovirus, adenovirus, coronaviruses, herpes simplex virus and Coxsackie virus
- **Purulent pharyngitis** - about 90% caused by S. pyogenes, in other cases by other bacteria, especially S. aureus, S. pneumoniae, C. diphtheria, B. pertussis, H. influenzae, etc.
- **Nasopharyngitis** - N. meningitidis, other bacteria of the genus Neisseria
- **Laryngitis** - parainfluenza virus, C. diphtheria, etc.

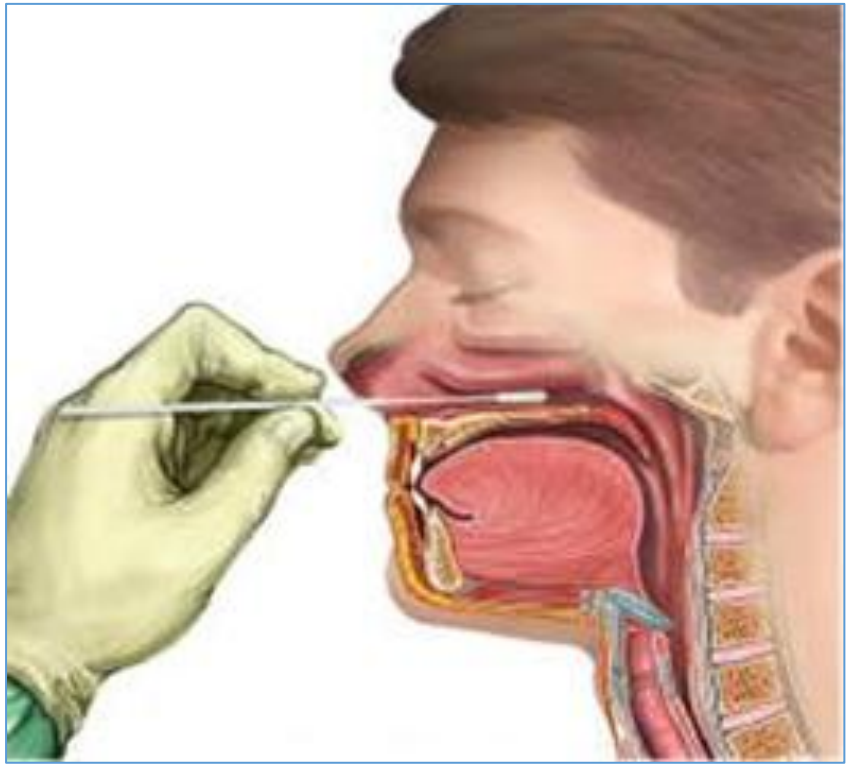
Principles of microbiological diagnosis of upper respiratory tract infections

- The specimen for microbiological diagnosis in upper respiratory tract diseases are obtained mainly by **sterile cotton swab**.



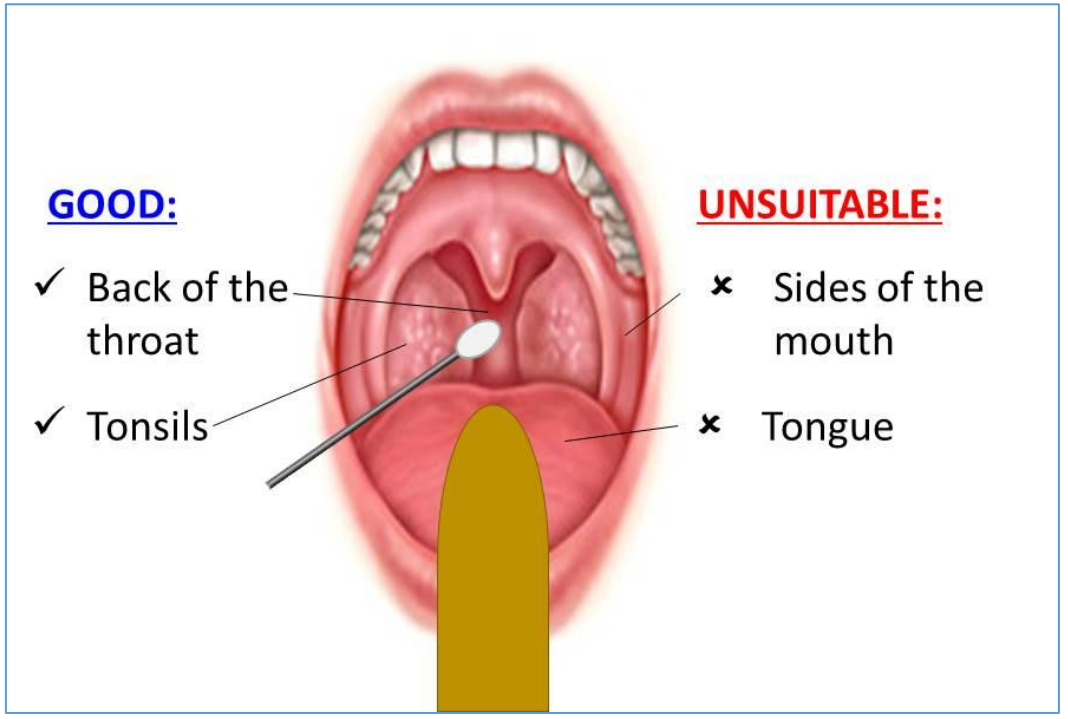
Obtaining material from the nasal cavity

- Specimen from the nasal cavity, is obtained by cotton swab imbedding first into the nasal cavity vertically and then horizontally



Obtaining material from the nasopharynx and throat

- Material is removed from the nasopharynx with a sterile posterior pharyngeal swab, and from the throat with a cotton swab soaked in sterile saline. In this case, the tongue should be fixed with a tounge depressor and the swab should not touch other areas of the oral mucosa.
- In some cases, pharyngeal lavage is performed. To do this, the patient is recommended to rinse with a sterile saline solution.



Principles of the diagnosis of upper respiratory tract infections

- Swabs used to obtain pathological material are delivered to the laboratory in a short time in sterile test tubes.
- Examples as a rule are inoculated on blood agar, chocolate agar, etc., incubated at 37°C for one day, cultured, identified, and tested for antibiotic susceptibility.
- The remaining specimen in the swabs is observed under the microscope after staining by Gram and Neisser methods.
- Virological tests are performed by inoculating the material into cell cultures and chicken embryos.

Microbiology of lower respiratory tract infections

- **Tracheitis and bronchitis -** H. influenzae b serotype, Neisseria, Morachella, streptococci and viruses (ortho- and paramyxoviruses, adenoviruses, coronaviruses). In addition to the causative agents of the acute process, in which the inflammatory process is chronic, there are other bacteria from the genus S. pneumoniae, S. aureus, P. aeruginosa, Klebsiella and Enterobacteriaceae and Candida
- **Pneumonia**
- Primary pneumonia occurs as a result of the entry of pathogenic microorganisms into lung tissue.
- In secondary pneumonia, the pathological process follows any pre-morbid condition called premorbid background (eg, circulatory disorders, immune deficiencies, immunodeficiency, etc.).
- In some cases, pneumonia is not a free disease, but manifests itself as a symptom of any disease. For example, pulmonary tuberculosis, systemic mycoses, ornithosis, Q-fever, legionellosis, etc. accompanied by pneumonia.

Pneumonia - causative microorganisms

- Pneumonia can be caused by a variety of microorganisms - bacteria, mycoplasmas, viruses, fungi and protozoa.
- Among the pathogens are Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Klebsiella pneumoniae, Mycobacterium tuberculosis.
- In relatively rare cases, pneumonia can be caused by enterobacteria, non-spore-forming anaerobes, Candida and other fungi

Pneumonia - causative microorganisms

- ***Bacterial pneumonia*** **-** with Gram-positive bacteria (S. pneumonia, S. pyogenes, S. aureus), Gram-negative bacteria (K. pneumoniae, E. coli).
- ***Viral pneumonia*** develops gradually and has an atypical course and is usually complicated by secondary bacterial pneumonia. Viral pneumonia is mainly caused by RS-virus, adenovirus, influenza and parainfluenza viruses. In relatively rare cases, it is caused by herpesviruses, rhinoviruses, measles, rubella, ECHO-viruses, Coxsackie viruses and coronaviruses.
- ***Atypical pneumonia*** is caused by Mycoplasma pneumoniae, Chlamydia psittaci, Legionella pneumophilia and viruses, most often pneumonia with typical (bacterial) pneumonia.

Principles of diagnosis of lower respiratory tract infections

- **Speciment** may be the sputum, bronchial lavage obtained by bronchoscopy and biopsy of the lungs, lung tissue puncture and aspirate, pleural effusion.
- For microbiological examination, sputum should be taken before the start of antibacterial treatment, or after its reception, after the time necessary for its elimination from the body..
- The morning portion of sputum is taken in a sterile container. Before taking sputum, the patient should rinse his mouth and brush his teeth with boiled water or a weak solution of antiseptics.
- Because bronchoscopy does not contaminate the upper respiratory tract with microflora, the examination of sputum taken by bronchoscopy is more informative.
- If the test is delayed, the sputum can be stored in the refrigerator at 40 ° C for no more than a few hours.

Microbiological examination of sputum

- Various methods are used for microbiological examination of sputum.
- **Microscopic method**. Purulent particles of sputum are identified after washing with an isotonic solution to release microflora of the upper respiratory tract.
- Sputum smears are stained with Gram and, if necessary, Ziehl-Neelsen methods (to detect mycobacteria).
- Microscopy of smears allows to estimate the nature and amount of microflora in sputum, as well as to determine the direction of bacteriological examination.

Microbiological examination of sputum

- It is relatively difficult to determine the etiological role of microorganisms derived from the fact that sputum is contaminated with microbes during passage through the upper respiratory tract and oral cavity.
- Bartlet's score is determined to determine the suitability of sputum for microbiological examination. Bartlett's score is calculated by sputum microscopy. To do this: 1) the number of neutrophils in a field of vision; 2) the presence of muscle fibers; 3) The number of epithelial cells in a field of vision is determined. The high number of neutrophils and muscle fibers is an indicator of the inflammatory process and thus the usefulness of sputum for microbiological examination. The large number of epithelial cells is not an indication of inflammation, but of contamination with saliva.
- A score of 1, 2, or 3 indicates active inflammation, and a score of 0 or lower indicates mild inflammation or contamination with saliva
- neutrophils in one visual field <10 = 0 points; 10-25 = + 1 point; > 25 = +2 points;
- presence of muscle fibers = + 1 point
- number of epithelial cells in a visual field: 10-25 = -1 point; > 25 = -2 points

Microbiological examination of sputum (bacteriological method)

- Purulent particles of sputum are inoculated into a number of nutrient media - blood agar, chocolate agar, differential-diagnostic media, media for anaerobes.
- When fungi are detected in the microscopic preparation, the sputum are inoculated into Saburo or other media for cultivation.
- When tuberculosis or mycoplasma infection is suspected, cultivation is carried out in appropriate media.

Microbiological examination of sputum (bacteriological method)

- Sputum taken with a bacteriological loop is inoculated by spreading it on the surface of a solid nutrient medium.
- 4-sector inoculation is more suitable, which allows to estimate the relative amount of microorganisms in the material and at the same time to obtain a pure culture.
- Samples are incubated for 24 to 48 hours, cultured, identified, and tested for antibiotic susceptiblity

Microbiological examination of sputum (bacteriological method)

- It is relatively difficult to determine the etiological role of microorganisms derived from the fact that sputum is contaminated with microbes during passage through the upper respiratory tract and oral cavity.
- Bacteriological examination of sputum is carried out by **quantitative method** to differentiate the causative microorganism from the microflora of the lower respiratory tract.

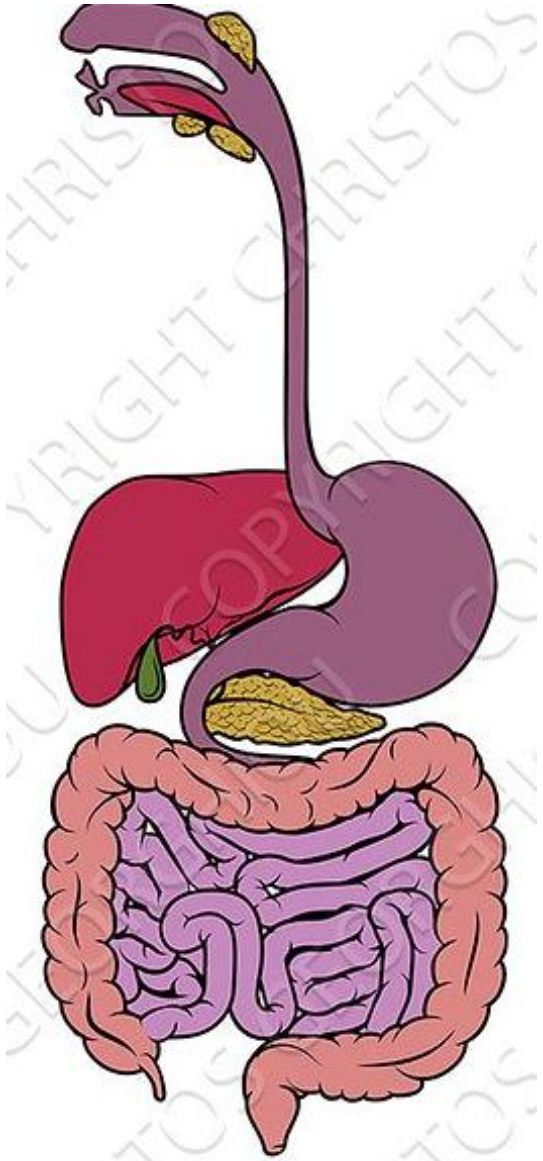
Microbiological examination of sputum (bacteriological method)

- To quantative examination 0.1 ml of carefully homogenized sputum is added in 0.9 ml of isotonic solution to prepare 10 times dilution. Then 0.1 ml of the obtained dilutions is taken and added to another test tube containing 0.9 ml of isotonic solution to obtain 10(2) dilutions.
- After diluting the sputum 10(6)-10(7) times according to this rule, 0.1 ml of the last dilutions is applied to the surface of the blood agar by rubbing it with a spatula. After incubation for 1-2 days at 370C, the results are recorded.
- Microorganisms obtained from dilution 10(6)-10(7) have an etiological role. The growth of microbes in small dilution is estimated as the contamination of sputum with the microflora of the upper respiratory tract.
- It should be considered that the amount of pathogens in the sputum may be low during antibacterial treatment.

Principles of diagnosis of lower respiratory tract infections (serological method)

- The serological method is mainly used in the diagnosis of viral pneumonia.
- Diagnostic indications are a four-fold or greater increase in antibody titers in double-serum samples taken at the beginning of the disease and two weeks later.
- In some infections, IgG and IgM are assigned to the causative agent by ELISA.

Microbiological diagnosis of gastrointestinal tract infections and dysbiosis



Microbiology of oral cavity diseases

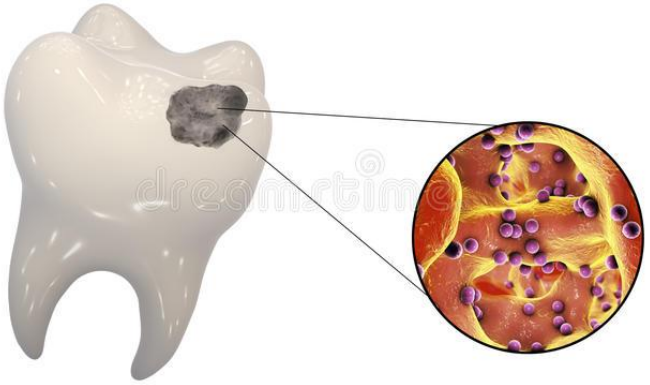
- Diseases of the soft tissues and teeth are distinguished among the diseases of the oral cavity.
- **Stomatitis** is an inflammation of the mucous membrane of the oral cavity. Catarrhal and gangrenous stomatitis are distinguished.
- *Catarrhal stomatitis* is a superficial inflammation of the mucous membrane - staphylococci, neisseria, haemophilus, opportunistic corynebacteria are involved
- *Gangrenous stomatitis* are mainly caused by anaerobes - fusobacteria, bacteroides, peptostreptococci, veilonellas, actinomyses, Vinsent's spirochetes, etc.

Microbiology of oral cavity diseases

- **Gingivitis** is an inflammation of the mucous membranes and tissues of the gums, mainly caused by microorganisms that are part of the gums, including spirochetes, bacteria of the genus Prevotella.
- *Vincent's gingivostomatitis* is characterized by acute hyperemia of the gums and the formation of foci of necrosis by fusobacteria (F.nucleatum), spirochetes (genes T.princelii). Staphylococci, streptococci, peptococci, velonella, actinomycetes, and bacteroids may play a role in the etiology of gingivitis.

Microbiology of oral cavity diseases

- **Caries** - *in the first stage* begins with the formation of spots (plaques) on the surface of tooth enamel. These are mainly gelatinous precipitates of high-molecular carbohydrates - glucans, which are adhesed by acid-forming bacteria. Glucans are mainly secreted by streptococci (S. mutans) (possibly in association with actinomycetes).
- *In the second stage*, streptococci and lactobacilli break down the carbohydrates in these spots to form large amounts of acid (pH <5.0). Such a high concentration of acids leads to demineralization process on an enamel and the formation of caries.



Microbiology of oral cavity diseases

- **Pulpitis** is an inflammation of the dental pulp, which usually occurs after caries as a result of the penetration of microorganisms into the pulp. It consists mainly of lactobacilli, streptococci, bacteroids, peptostreptococci, bacteroids, velonella, proteus and clostridia.
- **Periodontitis**. It occurs as a result of the penetration of microorganisms from the inflamed pulp into the soft and hard tissues surrounding the tooth - the periodontium.
- Microorganisms (streptococci and staphylococci, lactobacilli, fungi, veilonella, bacteroides) produse an enzymes (hyaluronidase, neyraminidase, collagenase) that destroy the connective tissue.

Microbiology of oral cavity diseases

- The entry of microorganisms into the tissues surrounding the teeth can result in periodontal pathology - **periodontitis** and **periodontitis**. Immunopathological processes play an important role in the pathogenesis of these diseases, accompanied by gingivitis and alveolar purulent inflammation.
- Periodontal pathology is an inflammatory-dystrophic process that occurs in the tissues surrounding the teeth. Anaerobes (Porphyromonas, Prevotella, Fusobacterium and Actinobacillus) play an important role in periodontal infections.

The microbiology of gastritis

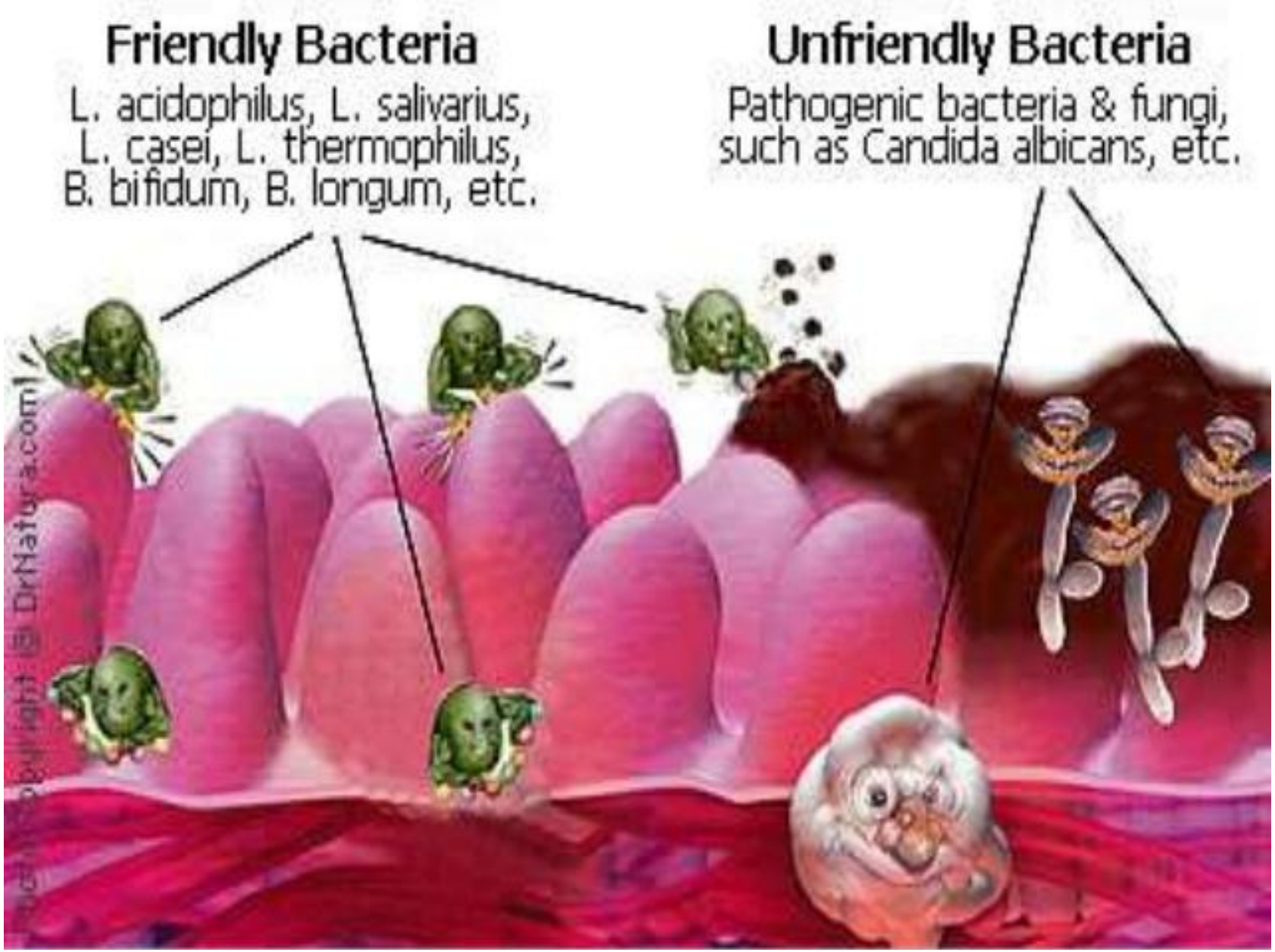
- Inflammation of the gastric mucosa can be caused by various exogenous and endogenous factors. In many cases, gastritis is manifested by inflammatory diseases of the intestine - *gastroenteritis* and *gastroenterocolitis*.
- Salmonella, which are the causative agents of food toxico-infections, play an important role in the etiology of acute gastritis.
- *H.pylori* causes intense inflammation in the mucous membrane of the stomach and duodenum. Acute infection manifests as gastroduodenitis, pain in the epigastric region and is accompanied by nausea. Later, chronic gastritis, gastric and duodenal ulcers may develop. The role of H. pylori in gastric cancer and gastric lymphoma has been confirmed.

Microbiology of acute intestinal infections

- Acute intestinal infections can be caused by bacteria, viruses and protozoa.
- The clinical manifestations of the disease are **enteritis, gastroenteritis, colitis, enterocolitis and gastroenterocolitis**. Diarrhea is one of the main clinical symptoms of acute intestinal infections.
- Bacteria that cause acute intestinal infections - E. coli, S. Typhi, S. Paratyphi A, S. Paratyphi B, Shigella, Vibrio cholerae, Campylobacter jejuni, Yersinia enterocolitica.
- Viruses that cause acute intestinal infections include Norwolk of the Caliciviridae family, as well as Sapporo viruses, adenoviruses, and rotavirus. Invasive protozoa that cause acute intestinal infections.
- Parasites of the genus Entamoeba histolytica, Balantidium coli, Giardia lamblia, Cryptosporidium, Isospora and Sarcocystis, Blastocystis
- The causative agents of food toxicoinfections are Clostridium botulinum, S.aureus, C.perfringens, B.cereus, S.Enteritidis, S.Typhimurium, S.Choleraceus

Dysbiosis and dysbacteriosis

- There is a certain balance between the representatives of the obligate and facultative microflora that make up the normal microflora of the body. This balance is primarily due to the antagonistic effect of the representatives of the obligate microflora on the facultative microflora.
- An imbalance between obligate and facultative microorganisms as a result of various factors leads to dysbiosis and dysbacteriosis.


Friendly Bacteria
L. acidophilus, L. salivarius,
L. casei, L. thermophilus,
B. bifidum, B. longum, etc.
Unfriendly Bacteria
Pathogenic bacteria & fungi,
such as Candida albicans, etc.

Dysbiosis and dysbacteriosis

- Dysbiosis is sometimes classified according to its location (oral cavity, intestines, uterine tract, etc.).
- The term dysbacteriosis is primarily understood as intestinal dysbacteriosis. The development of dysbacteriosis is associated with a reduction in the amount of obligate microflora, which is part of the normal microflora.
- As a result, multiplication of the opportunustic microorganisms - Proteus, Klebsiella, Enterobacter cloaceae, Citrobacter freundii, Serratia marcescens, Hafnia olvei, Morganella morqani, Providenca rettgeri, Pseudomonas aeruginosa, Staphylococcus aureus, Candida species of fungi and so on leds to appropriate diseases. Diseases caused by these microorganisms usually manifest themselves as intestinal infections.
- According to its etiology of dysbiosis, Candida, staphylococcus, proteus, etc. are distinguished.

Principles of microbiological diagnosis of gastrointestinal tract infections

- Stools, vomiting mass, gastric lavage, etc. were used as examination material.
- In some cases, especially in food toxico-infections, food and raw materials that cause the disease are examined.
- The material should be inspected in the first hours after acquisition; otherwise, a material preservative (phosphate-glycerin mixture, etc.) is placed.
- Microbiological examination of feces is carried out by microscopic, bacteriological, parasitological and virological methods.

Principles of microbiological diagnosis of gastrointestinal tract infections

- **Microscopic examination** is carried out by microscopy of native, sometimes Lugol-stained preparations of crushed drip preparations made from feces.
- Microscopic examination is used to assess the condition of digestion, normal microflora, signs of inflammation, as well as the diagnosis of protozoa and helminthiasis.
- The smear prepared from a suspension of faeces in a physiological solution can also be examined after staining by Gram and Sil-Nielsen methods.
- Examination of gram-stained smears reveals the presence of large gram-positive bacilli, such as C. difficile, staphylococci and Candida genus fungi.
- Sil-Nielsen staining reveals acid-fast Cryptosporidium and Isospora protozoa

Principles of microbiological diagnosis of gastrointestinal tract infections

- Bacteriological examination of feces is used for the diagnosis of dysbacteriosis, as well as the detection of bacteria that cause intestinal infections.
- Routine tests are performed by inoculation of a suspension of feces in a saline solution on nutrient medium.
- Fecal samples is performed by 4-sector inoculation with a bacteriological loop on the surface of the solid medium. This method allows to obtain a pure culture, as well as preliminary information about the amount of various microorganisms.

Principles of microbiological diagnosis of gastrointestinal tract infections

- In assessing the etiological role of the obtained cultures, it is important to determine their number, or rather the number of colonies (CFU) formed on the surface of the nutrient medium.
- To do this, it is important to consider the amount of material to be inoculated and the degree of dilutions. The amount of microorganisms is determined per 1 g of feces sample

The following criteria are used in the diagnosis of intestinal dysbiosis and dysbacteriosis:

- Total number of E coli in 1 g of feces sample;
- Relative amount of hemolytic E coli ;
- Presence and relative numbers of opportunistic microorganisms, including Proteus and Candida fungi:
- Total number of bifidobacteria, lactobacilli and bacteroides.

Principles of microbiological diagnosis of gastrointestinal tract infections

- Virological tests are used to detect Norwalk viruses, as well as adenoviruses. Freshly excreted feces or rectal tampons after storage in antibiotic media for 30 min. is inoculated on tissue cultures - the primary culture of the monkey kidney, the culture of human embryo, on the culture of fibroblasts.
- Immune electron microscopy, as well as PCR, is used to detect calciviruses and rotaviruses in feces.

Microbiology and principles of diagnosis of central nervous system infections

Pathogenesis and clinical forms of meningitis

- **Meningitis** is an inflammation of the meninges. The meninges are the three membranes that cover the brain and spinal cord. **Meningitis** can occur when fluid surrounding the meninges becomes infected. The most common causes of **meningitis** are viral and bacterial infections.
- **Leptomeningitis**, which is more commonly referred to as meningitis, represents inflammation of the subarachnoid space (i.e. arachnoid mater and pia mater) caused by an infectious or non-infectious process.
- **Arachnoiditis** is a pain disorder caused by the inflammation of the arachnoid, one of the membranes that surrounds and protects the nerves of the spinal cord. It is characterized by severe stinging, burning pain, and neurological problems.
- **Pachymeningitis** is a rare illness which can be shown by magnetic resonance imaging (MRI) to be a thickening of the intracranial dura mater, when associated with an infectious, malignant, or rheumatic systematic disease.
- Inflammatory changes during meningitis occur not only in the meninges and spinal cord, but also in the ventricles of the brain and vascular bundles, which is accompanied by hyperproduction and pressure of cerebrospinal fluid.
- During meningitis, changes in the composition of the cerebrospinal fluid occur: changes in the composition of the cell - an increase in the number of polymorphonuclear leukocytes, a decrease in the amount of glucose and an increase in the amount of protein.

Microbiology of meningitis

- Meningitis is a polyetiological disease that can be caused by most microorganisms.
- Meningitis is often caused by bacteria. *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae* are the main causative agents of bacterial meningitis in children.
- Occasionally *Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, E. coli, Klebsiella, Proteus, Pseudomonas, Listeria monocytogenesis, Gondialis, Brucella* and *Bruc*ella.

Clinical forms and microbiology of meningitis

- **Primary and secondary meningitis**
- **Acute and chronic meningitis.** Chronic meningitis is mainly caused by M. tuberculosis and fungi.
- Because most bacterial meningitis is purulent, they are sometimes called **purulent meningitis.** During some meningitis, it is difficult to detect microorganisms in the cerebrospinal fluid, or they are not detected at all. Such cases are characterized as **aseptic meningitis.** Aseptic meningitis is mainly caused by viruses, but meningitis caused by Mycobacterium tuberculosis, Leptospira, Cryptococcus, Tocoplasma gondii is also referred to as aseptic meningitis.
- When the inflammatory process involves the subcortical structures of the brain - brain tissue, the process becomes **meningoencephalitis**. Inflammation of the brain tissue is called **encephalitis**, and inflammation of the spinal cord is called **myelitis**. Encephalitis, myelitis, as well as encephalomyelitis are mainly caused by viruses.
- **Brain abscesses** are caused by anaerobic streptococci, Bacteroides, and post-traumatic abscesses are caused by more staphylococci and streptococci. In rare cases, abscesses caused by H.influenzae, A.israelii, N. asteroids and amoebae are also observed.

Bacterial (purulent) meningitis

- **The clinical picture** of all forms of bacterial (purulent) meningitis is characterized, above all, by high fever and meningeal syndrome.
- N. meningitis, S. pneumoniae and H. influenzae are the main causes of meningitis.
- E. coli is the main etiological agent of meningitis among bacteria of the family Enterobacteriaceae.
- Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, Klebsiella, Proteus, Pseudomonas, Listeria monocytogenes, Brucella and others. etiological meningitis is also found.

Viral meningitis and encephalitis

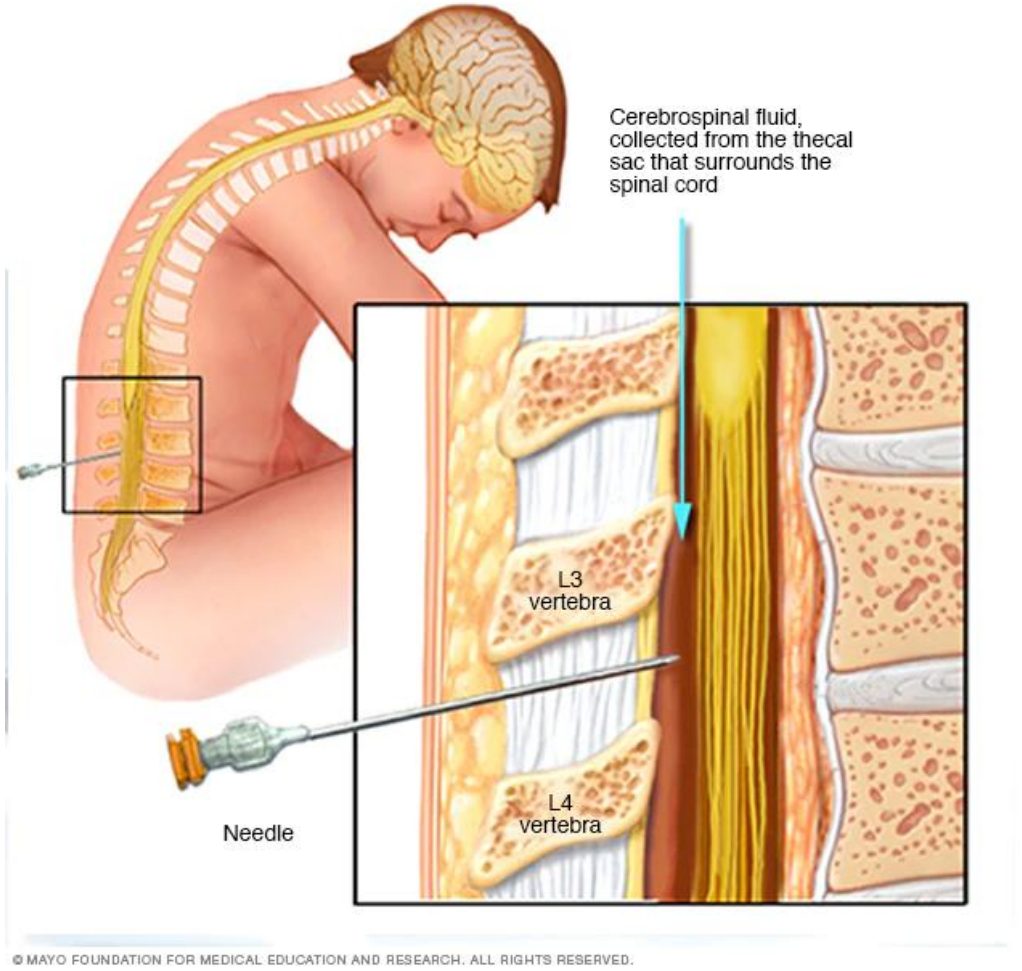
- Although viral meningitis and encephalitis present with symptoms of purulent meningitis, they usually present with mild clinical symptoms.
- Changes in the cerebrospinal fluid are weaker than in purulent meningitis, and bacteriological tests are usually negative (aseptic meningitis).
- In viral meningitis, the disease often manifests itself as meningoencephalitis, as the brain tissue is also involved in the process.
- Viral meningitis and encephalitis are most commonly caused by enteroviruses (poliovirus, Coxsackie- and ECHO viruses) and epidemic mumps virus. It can sometimes be caused by herpesviruses, including cytomegalovirus, measles, rabies, and arboviruses.

The fungal meningitis

- Systemic endemic mycoses, as well as opportunistic mycoses, may be associated with CNS damage.
- Recently, the role of fungi of the genus Candida, especially C. albicans, in the etiology of purulent meningitis has been increasing.
- Cryptococcal meningitis is observed in about 5-8% of AIDS patients.
- Zygomycota (Mucor, Rhizorus, Absidia, Rhizomucor etc.) cause rhinocerebral mucormycosis

Principles of diagnosis of central nervous system infections

- The main specimen for CNS infections is cerebrospinal fluid.
- The cerebrospinal fluid for the examination is obtained by a specialist doctor in strict accordance with the rules of asepsis.
- Due to the fact that the cerebrospinal fluid is sterile, it confirms the etiological diagnosis of any microorganism (excluding contamination!) Detected as a result of microbiological examination.
- Analysis of cerebrospinal fluid (CSF) may be important to indirectly determine the etiology of CNS infections.



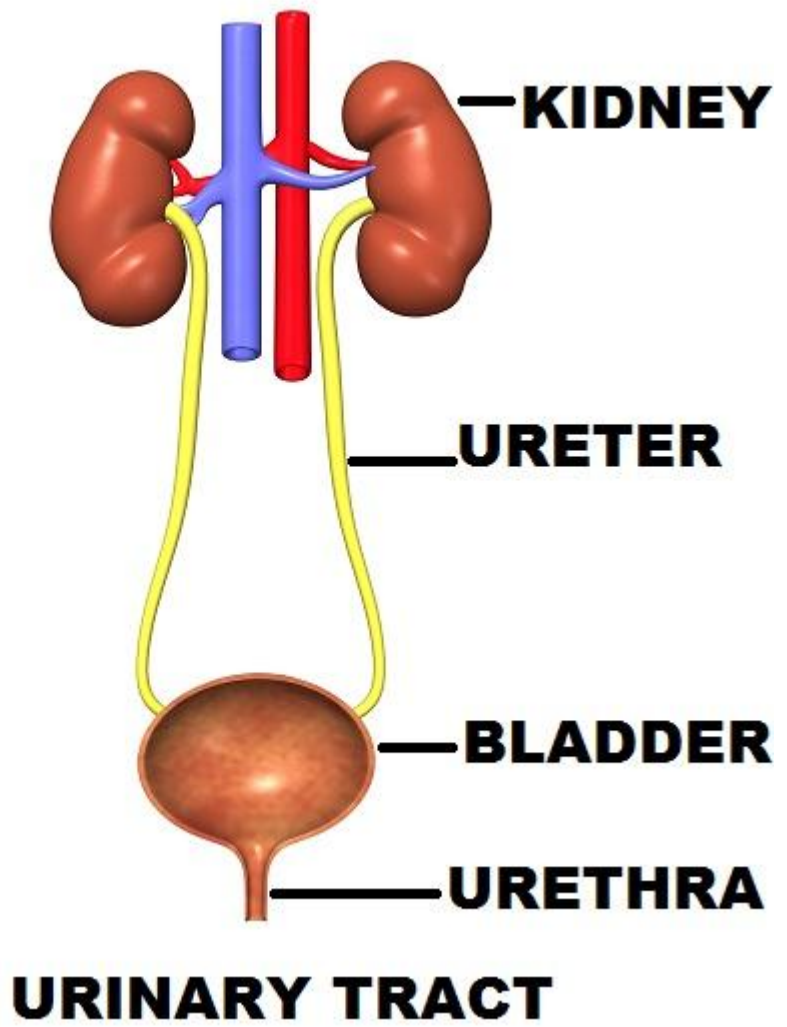
Microbiological examination of cerebrospinal fluid

- Microbiological examination of the cerebrospinal fluid (CSF) results in the preparation of ointments for microscopic examination of the centrifugal sediment, which is stained with methylene blue and Gram stain.
- If the cerebrospinal fluid is very turbid, it can be examined without centrifugation.
- The rest of the cerebrospinal fluid is used for bacteriological examination. Bacteriological examination involves inoculation of cerebrospinal fluid with various nutrient media – simple agar, glucose agar, blood agar, chocolate agar, Saburo.
- The obtained cultures are identified based on their morphological, cultural, biochemical and antigenic properties.
- In most cases, the acquisition of microorganisms from cerebrospinal fluid indicates their etiological role.

Microbiological examination of cerebrospinal fluid

- **Virological examinations** are performed during aseptic meningitis and are carried out by inoculation of cerebrospinal fluid into cell cultures, sometimes laboratory animals.
- **Serological tests.** The diagnosis of a four-fold or greater increase in the titer of antibodies to the virus in the blood via ELISA during periods of acute and convalescence of viral diseases of the CNS is confirmed.

Microbiology and principles of of diagnosis of urinary tract infections



Urinary tract: normal microflora

- Organs of the urinary system include the kidneys, renal pelvis, ureter, bladder and urethra.
- Normally, the kidneys, renal pelvis, ureter, and bladder are sterile, and no microorganisms are found.
- However, in the distal part of the urethra, some bacteria can be found, including Mycobacterium and common species of Mycoplasma, yeast-like fungi of Candida and other species.

Clinical forms of urinary tract infections

- The **clinical manifestations** of pathological processes in the urinary tract depend on the localization of the process.
- During **pyelonephritis**, fever, hematuria, leukocyturia, sometimes proteinuria, dysuric symptoms are observed.
- Pain in the groin area during **cystitis**, frequent painful, burning urine and transient hematuria, changes in the color, transparency and odor of urine, etc. observed.
- In **urethritis** dysuria, pain, dysuric symptoms, etc. is considered the main sign. Sometimes the symptoms of urethritis can be observed even in the absence of clinically significant bacteriuria. This condition, which is more common in women, especially sexually active women, is called **acute urethral syndrome**.

Microbiology of urinary tract infections

- The number of microorganisms in the urine of practically healthy people usually does not exceed 10 000 per 1 ml.
- Exceeding 100 000 microorganisms in 1 ml of urine is considered an indicator of urinary tract infection - **clinically significant bacteriuria**.
- The condition is called **asymptomatic bacteriuria** if it is not accompanied by clinical symptoms.
- Sometimes many diseases and pathological processes that are not related to the urinary tract, as well as manipulations can be accompanied by transient bacteriuria. In the absence of pathological processes in the urinary tract, bacteriuria is usually transient and is not detected in subsequent examinations.

Microbiological examination of urine

- Microbiological examination of urine is one of the main diagnostic methods in urinary tract infections.
- **The middle part of the morning urine** is taken in a sterile glass container for examination. If it is not possible to perform the test on time, the urine can be stored in a refrigerator at + 4 C for 24 hours.
- If the patient is unable to urinate freely, the urine is taken for examination by catheter or by puncturing the bladder from the superficial region.

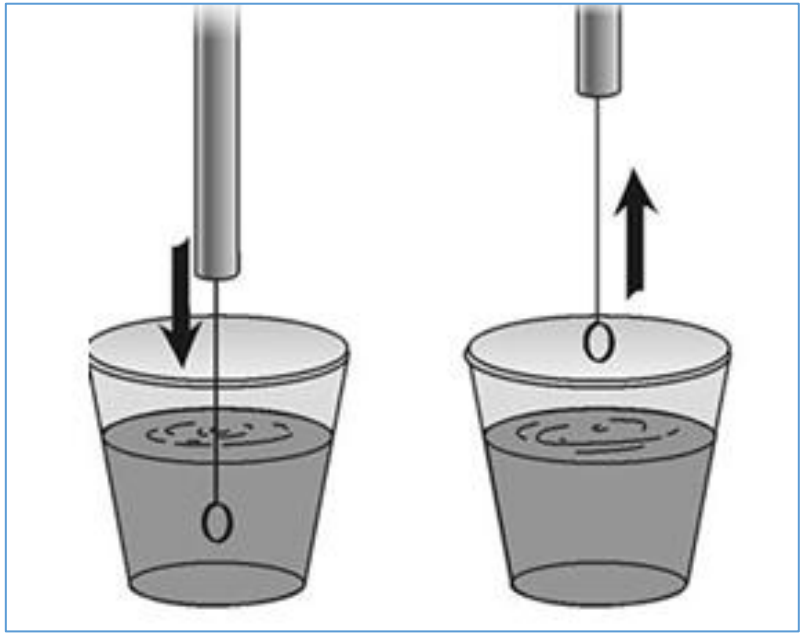
Microbiological examination of urine

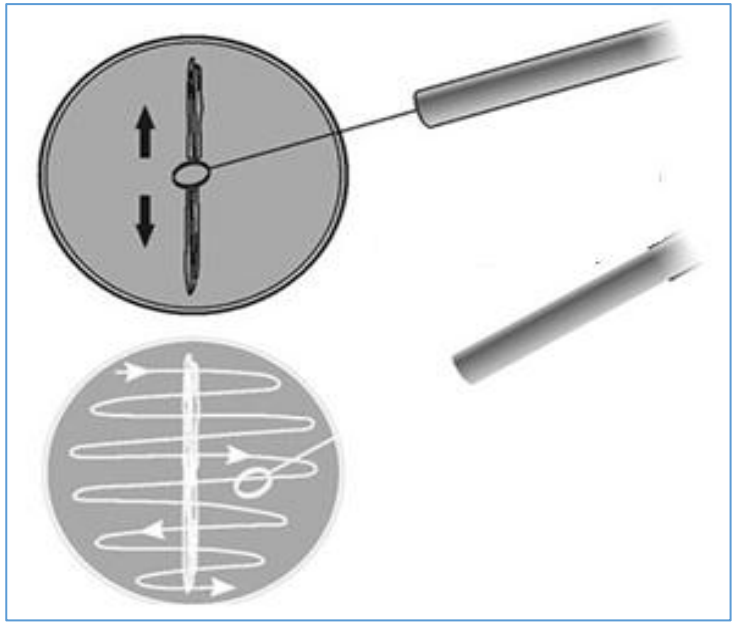
- **Microscopic examination.** Microscopy of urine sediment obtained after centrifugation reveals the presence of microorganisms, signs of inflammation, salts, etc.
- A smear is made from sediment usually is examined after staining by the Gram and Giemsa methods. However, microscopy of urine sediment does not allow to determine **the degree of bacteriuria**.
- The detection of one bacterial cell or leukocyte in each field of vision is equivalent to clinically significant bacteriuria.

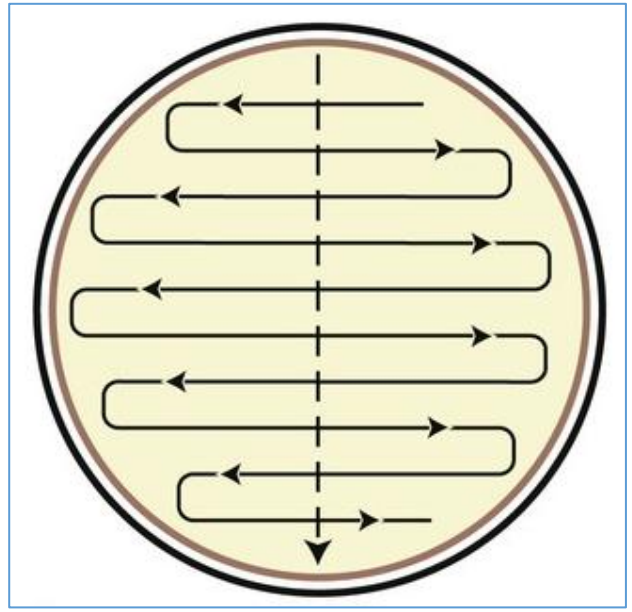
Assessment of bacteriuria

- The most common method is **calibrated loop**.
- The urine is carefully mixed and inoculated on a solid nutrient medium with a loop of known capacity. To do this, the loop is taken by inserting it vertically into the test material. The material in the loop is initially inoculated in a straight line along the diameter of the nutrient medium surface in the petri dish, and then perpendicular lines are drawn on this line.
- After incubation, the colonies are counted and the degree of bacteriuria is determined based on the capacity of the loop. For example, if the capacity of the loop is 0.001 ml, then the number of colonies is multiplied by 1000 and the number of bacteria in 1 ml of urine is calculated.
- The obtained pure culture is identified and its susceptibility to antibacterial drugs is determined.
- ***Detection of more than two types of microorganisms in the examined urine indicates incorrect sampling, in which case a repeat examination is performed***.

The inoculation procedure of urine on a solid nutrient medium using calibrated loop







Microbiology and principles of diagnosis of sexually transmitted diseases

The microbiology of sexually transmitted diseases

- In men, most sexually transmitted diseases enter the body through the mucous membrane of the urethra. In such cases, urethritis develops. *Neisseria gonorrhoeae, Trichomonas vaginalis, Chlamydia trachomatis, Mycoplasma hominis, Ureoplasma urealyti*cum are the main causes of urethritis.
- Urethritis is named, for example, gonococcal urethritis, chlamydial urethritis, etc. The term non-gonococcal urethritis is also used to distinguish other urethritis that is not related to gonorrhea.

The microbiology of sexually transmitted diseases

- ***Prostatitis*** **-** inflammation of the prostate gland can be of non-infectious and infectious origin. Infectious agents enter prostate tissue, usually through the urethra.
- In some cases, the causative agents of prostatitis are the causative agents of urinary tract infections, especially enterobacteria (E. coli, Klebsiella, Proteus, etc.), as well as genus P. ganida, S. aeruginosa.
- During sexually transmitted diseases, the causative agents usually enter the prostate gland through the urethra. During urethritis, the causative agents can damage the urethra, including the prostate gland.
- Thus, prostatitis caused by sexually transmitted microorganisms include gonococci, trichomonads, chlamydia, etc.

The microbiology of sexually transmitted diseases

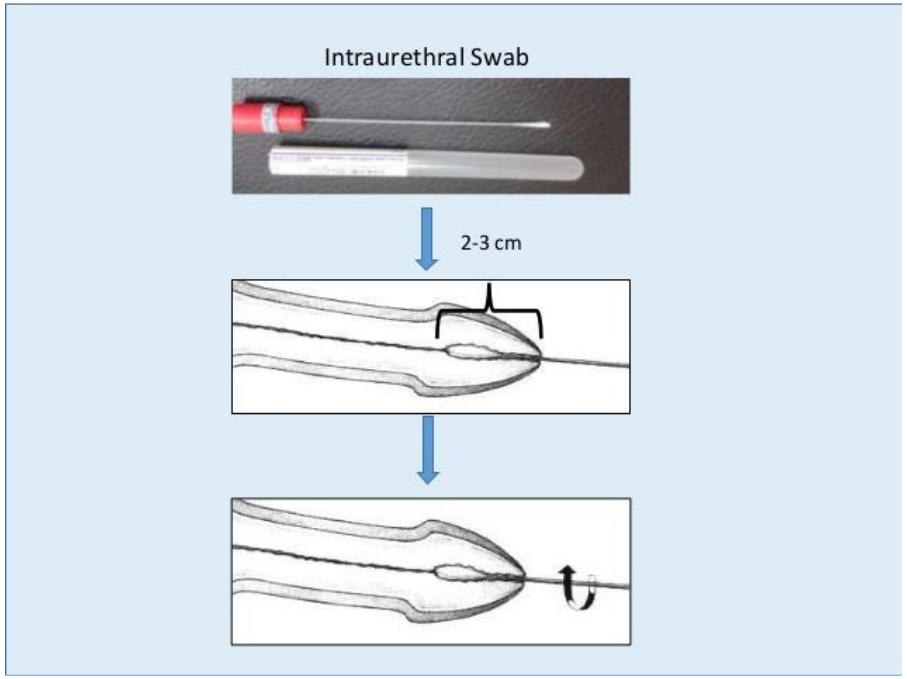
- **Infections of the female genital organs** can be conventionally divided into two groups: non-sexually transmitted diseases and mainly sexually transmitted diseases. Sometimes it is not possible to find such large differences between these infections.
- **Vulvovaginitis** is an inflammatory disease of the vulva and uterine tract, often occurring together. It is sometimes caused by facultative microflora in the vulva and uterus.
- Although Gardnerella and Vaginalis are the main causes of **bacterial vaginosis**, a number of anaerobic bacteria, such as Mobilincus, are involved in the development of the pathology.
- Gonococci, T. vaginalis, C. trachomatis, M. hominis, U. urealyticum can be the causative agents of vulvovaginitis caused by sexually transmitted infections.

The microbiology of sexually transmitted diseases

- The pathogens of some sexually transmitted diseases can cause vesicular lesions and superficial wounds directly from the skin.
- Vesiculosis lesions are most commonly caused by viruses, mainly herpes viruses. SHV type II infections are also known as genital herpes because they are sexually transmitted.
- Infectious lesions on the skin of the genitals can sometimes manifest themselves in the form of superficial wounds and erosions. During syphilis, a solid chancre is formed on the surface of the skin, where the Treponema pallidum enters. Damage caused by Haemophilus ducrey on the surface of the skin is in the form of a soft chancre - chancroid. In a rare disease called donovanosis (caused by Klebsiella granulomatis), there are red, non-purulent lesions with white margins.
- In genital infections, enlargement of the inguinal lymph nodes (buboes) is sometimes observed. It is characteristic of primary syphilis, genital herpes, lymphogranuloma venereum and soft chancre. AIDS is also characterized by generalized lymphadenopathy.

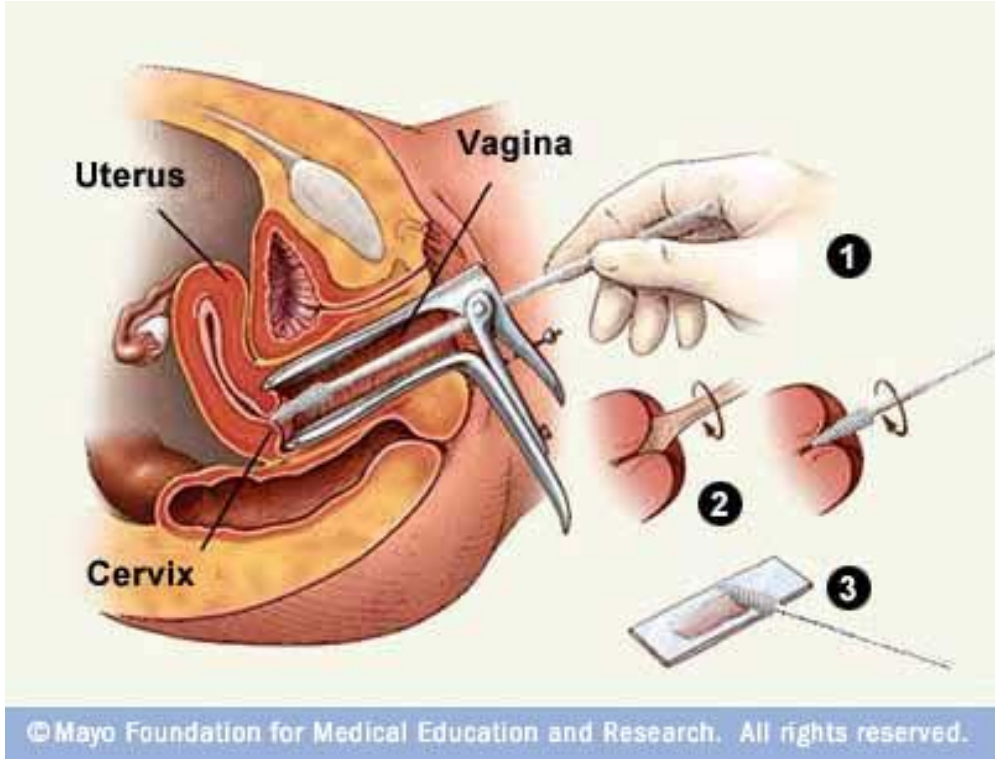
Principles of diagnosis of sexually transmitted diseases

- **Materials for investigation and rules of their collection**. Material is removed from the urethra for examination during urethritis. Material should be taken from the urethra in the morning before urination. Depending on the amount of urethral secretion, its removal is carried out in different ways.
- If there is **a lot of discharge**, the material can be removed with a bacteriological loop or a special cotton swab.
- When there is **a small amount of discharge**, especially when tested for chlamydia and other intracellular microorganisms, it is not the urethral discharge itself, but the pruritic tissue that comes from the mucous membrane of the urethra. Such material contains a large number of epithelial cells, which is necessary for microscopic examination in these cases. For this purpose, in addition to the urethral tampon, cytological brushes can be used.



Principles of diagnosis of sexually transmitted diseases

- ***Materials for investigation and rules of their collection.*** Material is removed from the vagina and vulva with a sterile cotton swab.
- When removing material from the vagina, gynecological mirrors are used to enhance the visualization effect, and fluid accumulated in the back of the uterus is obtained.
- In order to detect intracellular microorganisms, the material is removed from the mucous membrane with a cotton swab.
- The mucus is first removed from the cervical canal, the endocervical canal, with a sterile swab, then a special (urethral) tampon is inserted into the cervical canal and is rotated in the cervical canal .



Principles of diagnosis of sexually transmitted diseases

Rules for conducting microbiological examinations.

- A smear is made from the discharge taken from the urethra, which is examined microscopically after staining by the Gram method and methylene blue.
- This method is often used to diagnose gonococcal and trichomonad urethritis.
- It is possible to diagnose a bacterial vaginosis by identifying «clue cells" by microscopy of Gram-stained smear from vagina.

Principles of diagnosis of sexually transmitted diseases

- Ch.trachomatis, M.hominis / genitalium and U.urealyticum / parvum antigens can be detected in samples by IFR.
- During the IFR examination, the material taken with the tampon (rich in epithelial cells) is spread on a glass slide and fixed with acetone.
- After working with a specific fluorochrome-conjugated antibody, the preparation is carefully washed with a buffer solution, dried and examined under a fluorescent microscope.
- All the tools and reagents required for this procedure are included in special test systems for the determination of chlamydial antigens and are now available commercially.
- Recently, PCR, cultivation and microtest systems have been used more and more to detect these microorganisms.

Principles of diagnosis of sexually transmitted diseases

- Prostatitis, prostatovesukilitis, prostate gland secretion, ejaculate, as well as microbiological examination of urine are performed.
- The secretion of the prostate gland is usually obtained by massage after urination. Smear can be prepared and examined under microscope. A crushed drop smear also can be prepared.

Principles of diagnosis of sexually transmitted diseases

- **Cultural examinations.** The obtained specimen from the urethra, from the uterus, from the cervix, and so on, as well as aspirate materials, ejaculate, prostate secretion, urine are inoculated into the appropriate nutrient media when necessary.

Principles of diagnosis of sexually transmitted diseases

- **Serological tests**. Reactions based on the determination of specific antibodies in the blood serum are also used in the diagnosis of sexually transmitted diseases.
- In syphilis, VDRL and RPR tests allow the detection of non-specific antibodies. Only specific antitreponemal antibodies can be detected by TPHA and IFR.
- ELİSA is used in the diagnosis of herpes and SMV infections. Separate determination of IgM and IgG antibodies allows to differentiate between past and present infections.
- In the diagnosis of sexually transmitted infections, IFR is important in the detection of pathogens and their antigens in pathological material.

Principles of diagnosis of transplacental diseases

- In some cases, in microbiological practice, it is necessary to diagnose infections that damage the fetus through the plasenta, causing its death or abortion.
- Although some of these diseases are not sexually transmitted, the diagnosis of stillbirth, placenta, and amniotic fluid is important in their diagnosis. **Transplacental diseases include listeriosis, toxoplasmosis, rubella, cytomegalovirus, genital herpes, parvovirus infections, syphilis, AIDS, etc.**
- Reagents with specific IgM-antibody tests are currently available to identify TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes). Detection of IgM in the blood serum of newborns in these infections indicates neonatal infection. Thus, M antibodies are not transmitted to the fetus by the transplacental route.

Microbiological diagnosis of wound infections

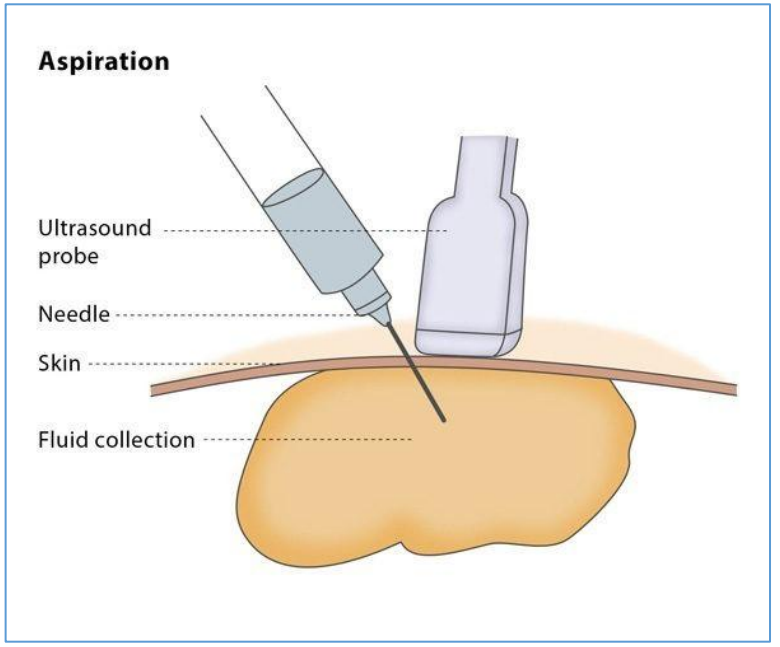
Wound infections

- Wound infections develop as a result of traumatic disruption of the integrity of the skin and mucous membranes.
- Wound infections manifest themselves in different ways, depending on the nature of the wound and its location, size, condition of the body, the nature of the agent that caused the injury, and so on.
- Various medical manipulations, especially after surgical interventions, can lead to the development of wound infections.

Microbiological diagnosis of wound infections (materials for investigation)

- In case of wound infections, the material is obtained from the depth of the wound with a sterile swab.
- Exudate from the cavities is obtained by a specialist doctor by puncture with a syringe in accordance with aseptic procedures and sent to the laboratory in anaerobic transport media.
- The abscess is punctured. Drainage material is obtained from a sterile syringe and transferred to a sterile test tube or anaerobic transport medium following aseptic procedures.
- Tissue fragments and foreign bodies taken from the wound area may also be examined.

Microbiological diagnosis of wound infections (materials for investigation)



Microbiological diagnosis of wound infections (microscopic examinations)

- **Smears** are made from the material taken from the wound contents with a cotton swab.
- If the exudate from the cavities (pleural exudate, empyema pus, synovial fluid, ascites fluid, etc.) is translucent, it is centrifuged, and the sediment is used for preparation of smear.
- When the exudate is purulent, smear are made directly from it. Morphology and quantity of microorganisms are recorded during microscopy after Gram staining.

Microbiological diagnosis of wound infections (cultural examinations)

- The wound contents are inoculated from the tampon into nutrient media – simple media, blood and glucose agar, Saburo medium, glucose broth, anaerobic media.
- Liquid samples are inoculated into a solid medium. Samples are incubated at 37 ° C under aerobic and anaerobic conditions. Obtained cultures are identified.
- When an association of microorganisms is obtained from the wound contents, the species that is quantitatively predominant is considered to be a microorganism with an etiological role.
- Any type of microorganism obtained from sterile body cavities and from the depths of purulent wounds, is considered to be a microorganism with an etiological role.

Microbiological examination of wound tissue

- Pieces of tissue are crushed with a sterile lancet, 1 gram of tissue is "dissolved" in 1 ml of food broth.
- The ten-time dilution are prepared from the obtained dilution, 0.1 ml of each dilution is inoculated into solid nutrient media with a spatula.
- Based on the number of colonies growing after incubation and the degree of dilution, the number of microorganisms per 1 g of tissue is calculated.
- The presence of 100 000 or more microorganisms in 1 g of wound tissue is a diagnostic indicator.

The microbiology and principles of diagnosis of septic infections

Bacteremia and sepsis

- The pathological processes associated with the entry and multiply of microorganisms in the blood can manifest themselves as bacteremia and sepsis.
- **Bacteremia** (viremia, fungemia, parasitemia, etc.) refers to the entry of microorganisms into the blood. Microorganisms can enter only through exogenous routes (for example, as a result of trauma) or from sources of infection in the body. The latter case is observed during bacterial infections.
- During **sepsis** (Latin, sepsis - pus), microorganisms stay in the blood for a long time and multiply there.

Infections that accompanied by Bacteremia

- **Bacteremia** can be caused by most bacteria in practice. Bacteremia caused by gram-negative and gram-positive bacteria differ in certain features.
- **Gram-negative bacteremia** is mainly caused by Enterobacteriaceae (E.coli, Klebsiella, Proteus, Serratia, Proteus, Enterobacter, etc.) and P.aeruginosa. Infections are more likely to enter the gastrointestinal tract, urogenital tract and skin.
- **Gram-positive bacteremia** is mainly caused by S. aureus and coagulase-negative staphylococci (S. epidermis and S. saprophyticus). Staphylococcal bacteremia is caused by the skin, as well as any source of infection in the body.

Septic infections

- The main causative agents of bacteroid-induced septicemia, *Bacteroides fragilis* and *Prevotella melaninogenica*, are often found to be associated with other bacteria.
- *P.melaninogenica* enters mainly from the oral cavity, and B.fragilis only from the primary hearth in the gastrointestinal tract.
- During septicemia caused by clostridia, the causative agents are often identified in association with other anaerobic and aerobic bacteria. The main causative agent, *C.perfringens*, enters the intestinal tract and bile ducts, and in some cases, after abortions, from the mucous membranes of the uterus.

Principles of diagnosis of septic infections

- Microbiological diagnosis is based on **bacteriological examination of blood**.
- Blood should be taken from the elbow vein in strict adherence to aseptic conditions before the start of antibacterial treatment or after a certain period of time for the elimination of the drug from the body.
- Bacteriological examination of blood is based on the acquisition of the pathogen from the blood - the obtaining of **hemoculture**.

Bacteriological examination of blood

- For this purpose, the blood is immediately transferred to a liquid nutrient medium, and in its absence, to a sterile vial containing reagents (sodium citrate, heparin, etc.) that prevent the blood from clotting.
- To neutralize the effects of bactericidal factors, the blood is inoculated into a nutrient medium 5-10 times the volume of taken blood (usually 5-10 ml of blood is inoculated into 50-100 ml of nutrient medium). Special nutrient media are used when typhoid fiver and other infectious diseases are suspected.
- Samples are incubated at 37 ° C for 10 days under daily observation.
- When grown in a nutrient medium (broth turbidity, sedimentation, etc.), it is transferred to blood agar, pure culture is obtained, identified and determined susceptiblity to antibiotics.

Bacteriological examination of blood: interpretation

- Obtaining of any microorganism from the blood is assessed as bacteremia and sepsis.
- A single blood test does not always provide a hemoculture.
- If the result is negative, it should be examined at least three times.