AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

LESSON 21.

Microbiological diagnosis of infections of the central nervous system and urogenital system

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

Discussed questions:

1. Central nervous system, brief anatomical and physiological information
2. Inflammatory diseases of the brain and meninges, meningitis, encephalitis, causative microorganisms.
3. Microbiological examinations conducted during inflammatory diseases of the brain and meninges.
4. Liquor collection, shipping and examination methods.
5. Organs of the urinary system, brief anatomical and physiological information.
6. Normal microflora of the urinary tract, inflammatory diseases and their causative agents.
7. Microbiological examination of urine. Bacteruria, its determination and evaluation. Diagnostic value of microscopic examination of urine
8. Genital organs, brief anatomical and physiological information.
9. Sexually transmitted diseases.
10. Inflammatory diseases of female genital organs, material collection and examination rules.
11. Understanding of TORCH infections. Transplacental diseases and their diagnosis.
12. Inflammatory diseases of male genital organs, material collection and examination rules

Purpose of the lesson:

- To acquaint students with the microbiological examinations performed during inflammatory diseases of the brain and meninges, their causes, meningitis and encephalitis. Also, to acquaint them with the normal microflora of the genitourinary tract, inflammatory diseases and their causative agents, as well as the principles of microbiological diagnosis of these diseases.

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

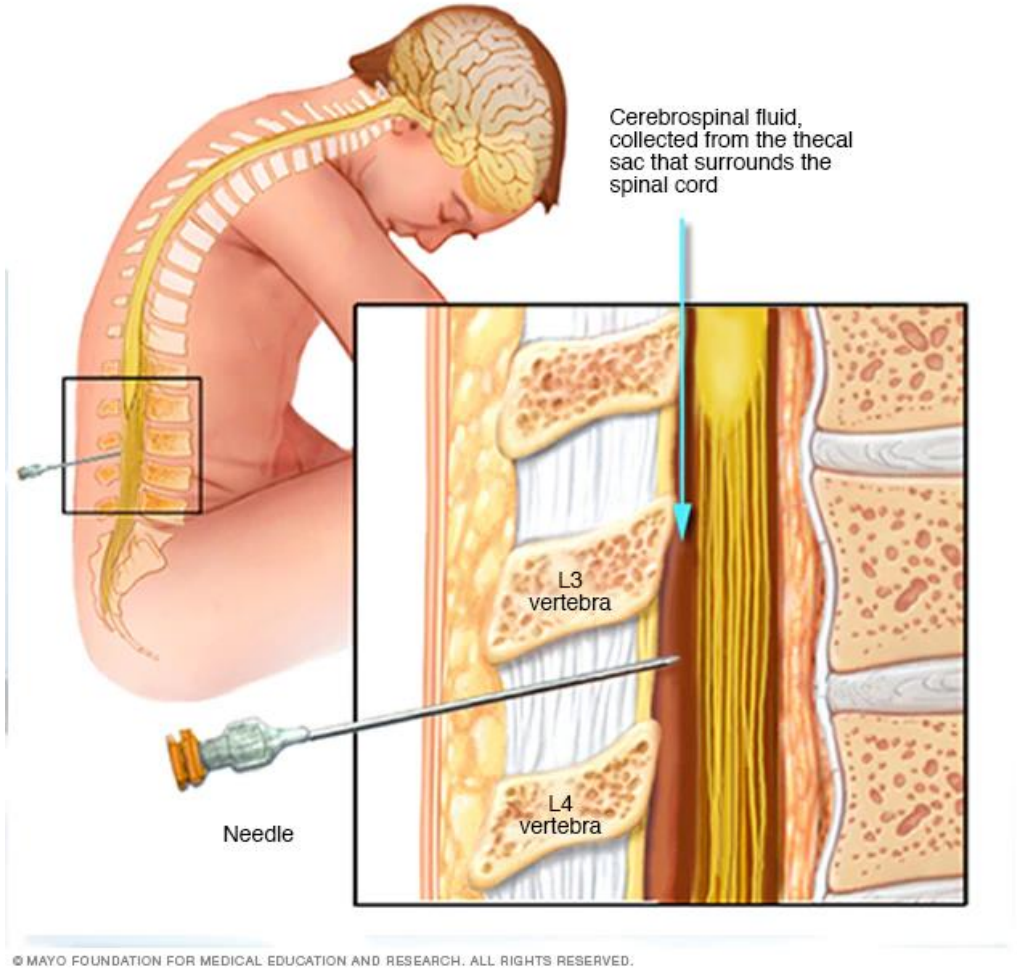
- Although viral meningitis and encephalitis present with symptoms of purulent meningitis, they usually present with mild clinical symptoms.
- Changes in the cerebrospinal fluid are weaker than in purulent meningitis, and bacteriological tests are usually negative (aseptic meningitis).
- In viral meningitis, 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.



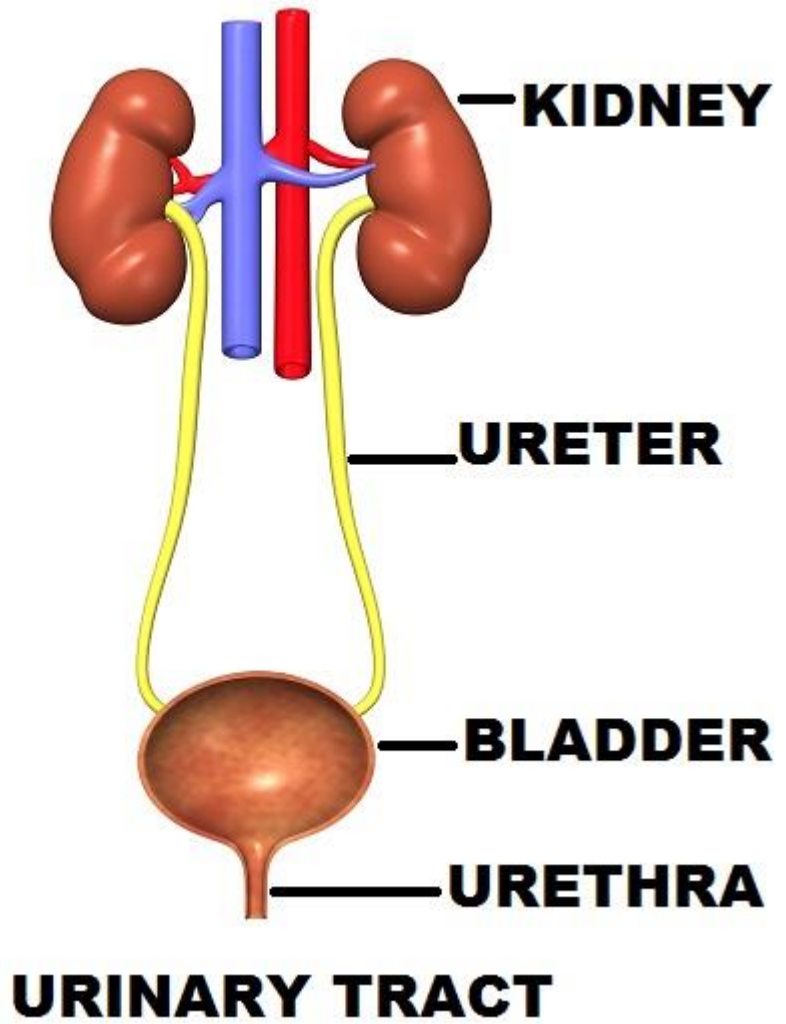
Microbiological examination of cerebrospinal fluid

- 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.

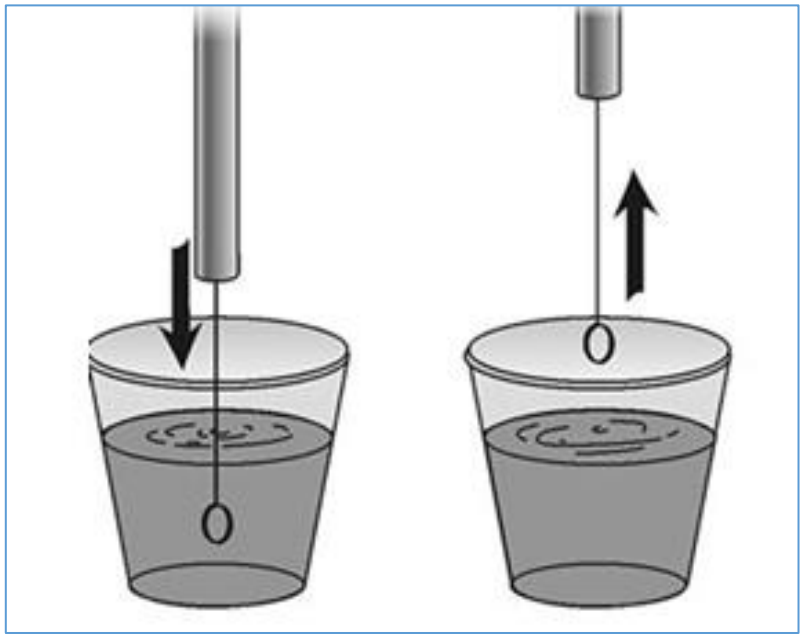
Microbiological examination of urine

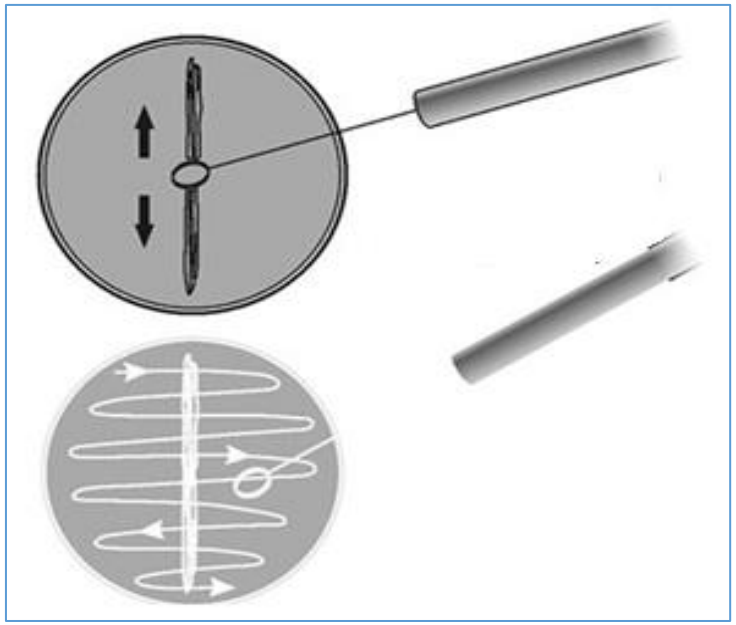
- **Microscopic examination.** Microscopy of urine sediment obtained after centrifugation reveals the presence of microorganisms, signs of inflammation, salts, etc.
- A smear is made from sediment usually is examined after staining by the Gram and Giemsa methods. However, microscopy of urine sediment 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.

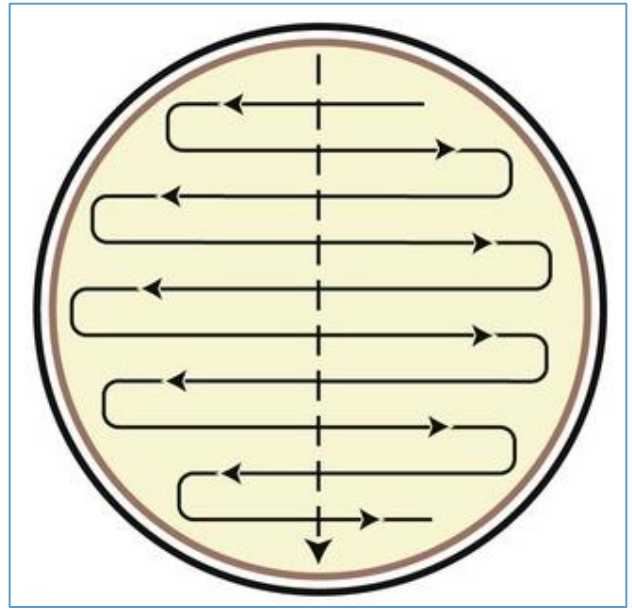
Assessment of bacteriuria

- 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

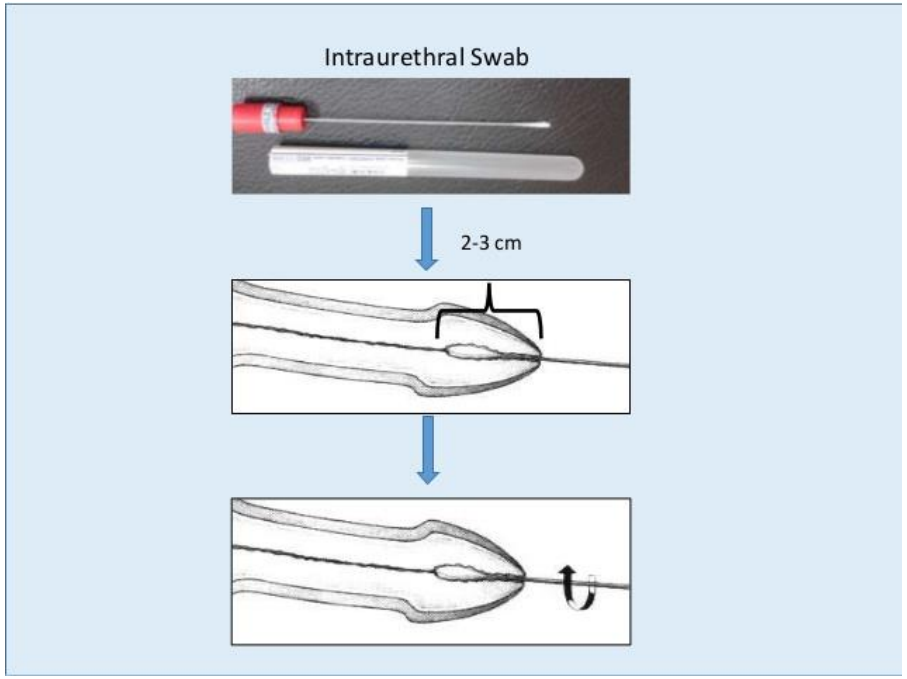
- **Infections of the female genital organs** can be conventionally divided into two groups: non-sexually transmitted diseases and mainly sexually transmitted diseases. Sometimes it is not possible to find such large differences between these infections.
- **Vulvovaginitis** is an inflammatory disease 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.

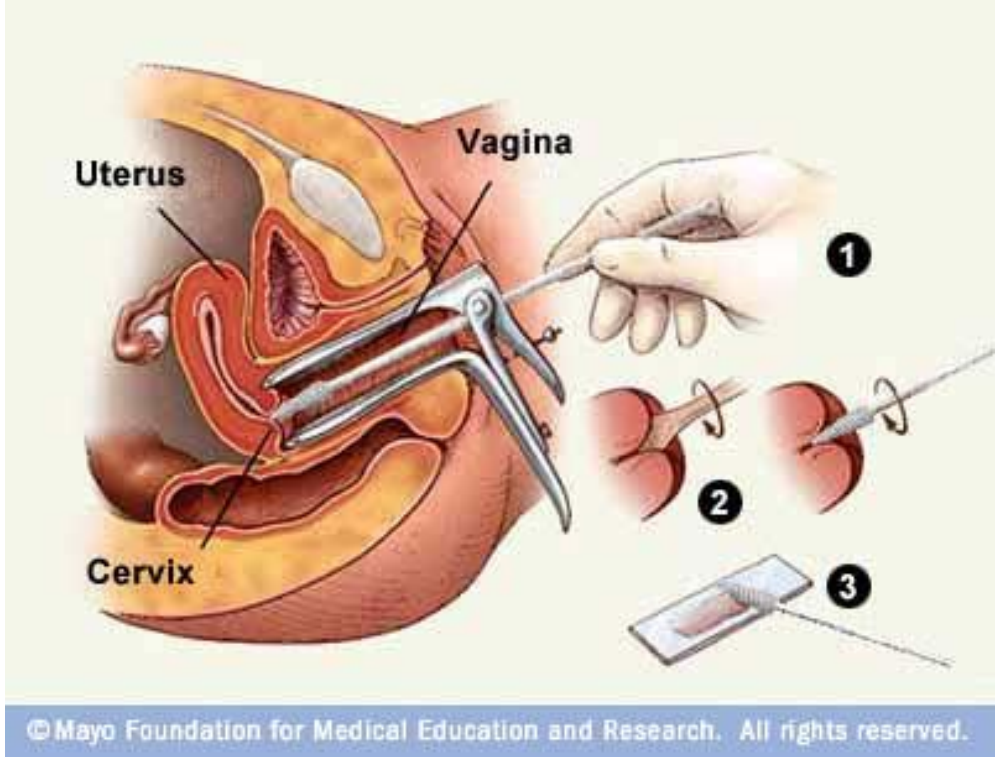
Principles of diagnosis of sexually transmitted diseases

- **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.