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Department microbiology

METHODOLOGICAL MATERIALS

on discipline - microbiology, virology, immunology

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Vladikavkaz

SCROLL METHODOLOGICAL MATERIALS:

1. Educational and methodical benefits for students on specialties "General Medicine" (part 1).
2. Educational and methodical benefits for students on specialties "General Medicine" (part 2).
3. Educational and methodical benefits for teachers on specialties "General Medicine" (part 1).
4. Educational and methodical benefits for teachers on specialties "General Medicine" (part 2).
5. Test tasks for students of the medical faculty on general microbiology.
6. Test tasks for students of the medical faculty on private microbiology.

**FEDERAL STATE BUDGETARY EDUCATIONAL INSTITUTION HIGHER EDUCATION
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**COLLECTION METHODOLOGICAL DEVELOPMENT
ON MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY FOR
STUDENTS OF MEDICAL FACULTY**

SPRING SEMESTER

Vladikavkaz

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Main purpose developments - methodological assistance to students to each practical occupation in spring semester. Directions drawn up in accordance with Federal publiceducational standard Supreme and professional education.

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Practical occupation No. 1.

Theme: Morphology microbes. Microscopic method research.

Educational goal:

1. Explore morphology individual representatives bacteria.
2. master technology microscopy.
3. master simple method coloring microorganisms.

The student must know:

1. Device and equipment microbiological laboratories, modework and appointment.
2. classification bacteria.
3. Structure microscope.
4. Methods microscopic research.
5. Technique microscopic research.

The student must be able to:

1. cook smear from clean culture, paint simple way.
2. microscoping With immersion system.

Plan lessons:

1. Familiarity with the rules of work and the basics of safety in microbiological laboratories.
2. device and equipment microbiological laboratory, working mode and appointment.
3. Classification bacteria.
4. Morphology bacteria, methods study (light, darkfield, phase contrast, electronic microscopy).
5. Stages cooking smear.
6. Simple method coloring bacteria.
7. Cooking smears from staphylococcus cultures and coli, coloring simple method.
8. Demonstration of preparations from micrococci, diplococci, tetracocci, sarcin, staphylococcus, streptococci, intestinal sticks, bacillus, vibrios.

Independent Job students

1. Cooking smear and coloring simple method (under leadership teacher).
2. Development technology microscopy. microscopic studying morphology bacteria:
3. View demonstration smear from clean culture staphylococcus (Staphylococcus aureus). Coloring gentian violet.
4. Viewing a demonstration smear from pure intestinal culture sticks (E. coli). Coloring — water fuchsin.
5. Decor protocol research.

INFORMATIONAL MATERIL ON THEME

1. Technics cooking smears.

Smears cook on fat free subject glasses, previously outlining with a pencil on the glass, the place of the future smear on the opposite side of the subject glass. At growth bacteria on liquid nutritional environment, material take sterile bacterial loop, applied to glass and rubbed over the outlined area. When growth bacteria on dense nutritional environment, on subject glass previously inflict loop a drop of water and material rubbed.

2. The bacterial loop is sterilized in burner flame. The prepared smear is dried in air or by holding high above flame spirit lamps.

3. After this a drug fix, for what smear side, where No material, three times through the middle of the burner flame. Fixation allows you to kill germs, attach them to glass and, finally, killed microbes stained better, how alive.

2. Technics simple methods coloring

The staining of bacteria aims to make them sharply different from the background, which allows explore under microscope them morphology and structure. AT microbiology use simple and complex methods coloring drugs.

At A SIMPLE METHOD of coloring, a smear is stained with any one dye, for example, water magenta (2-3 min.) or methylene blue (2-3 min.), washed water, dry up and microscopic.

3. Technics microscopy

Due to the very small size of bacteria, the study of their morphology is possible only at big magnification, achieved at help immersion oils, which allows you to create a single system between the glass slide and a special, x 90-fold (with black stripe) lens.

At microscopy painted objects necessary create bright lighting With help concave mirror, raised condenser and fully open diaphragm.

A drop of immersion oil is applied to the area of the smear on the glass lying on the table. The glass is then transferred to the microscope stage. The immersion lens is immersed in oil carefully, under the control of the eye, until there is obvious contact between the lens and the oil. Then the lens raise, not bringing out from drops oils and looking in eyepiece for finding object research ("fields vision"). Clear image drug achieved regulation first with a macro screw (for detecting an object), and then with a micro screw for adjustment sharpness Images.

Morphology major forms bacteria

known four main forms bacteria:

1. Cocci **are** microbes of a rounded shape, having a diameter of 1-2c. They differ between yourself on mutual location individual cells, which depends from way them division. If on graduation division cocci are separated on individual balloons, are obtained single cells cocci - Micrococcus.

2. Group from two cocci wears title diplococcus -Diplococcus (meningococcus, gonococcus have resemblance With beans, and lanceolate shape — Pneumococcus).

3.If the division of cocci occurs in only one direction and the resulting cocci do not are separated, then a thread of balls is obtained in the form of a chain, more or less long in dependencies from numbers cocci- Streptococcus.

4.When dividing in two mutually perpendicular directions, combinations arise along four cocci-Tetracoccus.

5.If division going on in three mutually perpendicular directions, cocci connect in in the form of packages (cubes) and receive title — Sarcina.

6.Sharing in various directions without special correctness cocci form disorderly clusters cells, reminiscent of grape bunches, why they and received title Staphylococcus.

Rod-shaped microorganisms are represented by the most numerous and diverse a group of bacteria. In the classification of rod-shaped forms, it is customary to refer to bacilli and clostridia are those sticks that are able to form spores, and those that are incapable of spore formation called bacteria. rod-shaped forms differ on size, location — one by one, couples, chain, disorderly and under angle. outline ends — rounded, chopped off, thickened, pointed.

Convolutd forms - spirilla and spirochetes having the appearance of corkscrew-shaped convoluted cells. Pathogenic spirillums include the causative agent of sodoku (rat bite disease). To the twisted same relate campylobacter, having curves How at flying wings seagulls.

Spirochetes — thin, long, curved (spiral forms) bacteria, different from spirilla mobility, conditioned flexion changes cells. Spirochetes presented three childbirth pathogenic for person: Treponema, Borrelia, Leptospira.

Methods diagnostics infectious diseases

1. *Microscopic method* lies in cooking drugs (native or painted simple or complex methods) from researched material and them microscopy With application various species microscopic technology (light, dark-field, phase-contrast, electronic). In bacteriology, the microscopic method called bacterioscopic in virology — viroscopy.

2. *Cultural method* is in sowing researched material on artificial nutrient media for the purpose of isolating and identifying a pure culture pathogens. AT bacteriology cultural method received title bacteriological, a in virology - virological.

3. *The biological method* (experimental or bioassay) is to infect researched material sensitive or others biological objects (chicken embryos, cell cultures). It is used to isolate a pure culture of the pathogen, determining the type of toxin, determining the activity of antimicrobial chemotherapeutic drugs.

4. *Serological method* - consists in determining the titer of antibodies in the blood serum the patient, less often - in the detection of microbial antigen in the test material. With this goal are used reactions immunity.

5. *Allergic method* is in identifying infectious allergies (GZT) on diagnostic microbial a drug — allergen. FROM this goal put skin allergic tests With relevant allergens.

An object study medical microbiological laboratories — pathogenic biological agents — pathogenic for human microorganisms (viruses, bacteria, mushrooms, protozoa). AT accordance With types microorganisms allocate: bacteriological, virological, mycological, protozoological laboratories. The regulation of working conditions with pathogens of infectious diseases was carried out in according to the degree of danger of microorganisms to humans. Allocate four groups pathogens.

Group 1: pathogens especially dangerous infections: plague, natural smallpox, fever Lassa, Ebola.

Group 2: exciters highly contagious bacterial fungal and viral infections: Siberian ulcer, cholera, fever, loose typhus, rabies.

Group 3: pathogens bacterial fungal, viral and protozoan nosological forms (whooping cough, malaria, polio, leishmaniasis).

Group 4: causative agents of bacterial, viral, fungal diseases (pseudomonal infection, amoebiasis, aspergillosis).

Microbiological laboratories work With PBA With pathogens especially dangerous infections (group 1 and 2). The special mode is maximally isolated by the individual and public risk.

TEST TASKS FOR CHECKS KNOWLEDGE

Specify one correct answer:

1. Which scientist is associated with the discovery of the essence of fermentation (1857), microbial conditionality and contagiousness infectious diseases (1881) manufacturing methods vaccines and ways prevention chicken cholera Siberian ulcers and rabies (1882-1885)?

- a) Leeuwenhoek
- b) Mechnikov
- c) Koch

d) Pasteur

2. Microbe - this is:

- a) a pre-cellular living being;
- b) organism certain type;
- c) a single-celled creature, invisible to the naked eye;
- d) infectious protein particle;
- e) unicellular organism.

3. A bacterium is:

- a) virus;
- b) unicellular creature certain kind, pertaining to prokaryotes;
- c) a single-celled creature of a certain species, belonging to eukaryotes;
- d) organism certain type;
- e) unicellular organism.

4. bacilli — this is:

- a) cocci, generators disputes;
- b) rods that do not form spores;
- c) rods that form spores;
- d) tortuous forms.

5. Alive maybe reckon system, able to:

- a) exchange substances With environmental environment;
- b) maintaining its structural organization;
- c) multiplication their structural components;
- d) reproduction and implementation genetic programs;
- e) metabolism.

Practical occupation No. 2.

Theme: Morphology microbes. Microscopic method research.

Control occupation.

Educational goal:

- 1. Explore individual structures prokaryotic cells.
- 2. master complex methods coloring microbes.

The student must know:

- 1. Peculiarity buildings bacterial cells.
- 2. The functional significance of the various components of the prokaryotic cells.
- 3. Sophisticated staining methods (Gram, Ziehl-Nelsen, Ozheshko, Burri-Gins, Neisser).

The student must be able to:

- 1. Prepare a smear from a pure culture of bacteria E. coli, S. aureus and paint on method Gram.
- 2. microscoping smear.
- 3. Be able to cook drugs "hanging" and "crushed" drops.

Plan lessons:

- 1. Structure and chemical composition bacterial cells.
- 2. Peculiarities buildings bacterial cells.
- 3. Complex methods coloring, goals them use (methods Grama, Tsilya-Nelsen, Ozheshko, Ginsa Burri, Neisser).
- 4. Demonstration painted drugs on methods Grama, Burri Gins, Neisser, Ozheshko, Ziel-Nielsen.

5. Studying mobility bacteria, cooking drugs "hanging" and "crushed" a drop.

Independent Job students

1. cook mixed smear from pure cultures staphylococcus and intestinal sticks. Coloring with water fuchsin.
2. cook mixed smear from clean culture staphylococcus and intestinal sticks. coloring by Gram.
3. cook drugs "hanging" and "crushed" a drop.
4. Decor protocol research.

INFORMATIONAL MATERIL ON THEME

Technics difficult methods coloring

Complex ways coloring include sequential drawing on a drug dyes that differ in chemical composition and color, mordants and differentiating substances. it allows previously differentiate microbes (*differential- diagnostic ways*) and reveal certain structures cells (*special ways*).

1. Way coloring on Gramu

Coloring on Gramu is important diagnostic sign identification bacteria. AT result coloring on Gramu Everybody bacteria share on two groups; gram- positive (blue colors) and gram negative (red colors).

Technics coloring according to the method Grama

1. Fixed smear put on bacteriological bridge and cover stripe filter paper impregnated with gentian solution -violet on a paper strip inflict water. Through 2 minutes stripe are removed.
2. Not flushing a drug water, inflict solution Lugol on 1 minute. Then solution drained.
3. A drug discolor alcohol 20-30 seconds (before discharge purple trickle paints).
4. A drug washed water.
5. coloring water magenta — 2 minutes.
6. A drug washed water.
7. Dry up on air or filtering paper.

2. Way coloring according to Ziel-Nelsen

Applies for detection some germs, rich lipids (for example, pathogen tuberculosis, leprosy and etc.)

1. For staining use concentrated solution carbolic magenta Tsilya. FROM goal improvements penetration dye in cage a drug With imposed on him stripe filtering paper and dye warm up above flame burner three times before appearance pair.
2. Then drug discolor five% solution sulfuric acid, previously removing filtering paper.
3. washed water.
4. Finishing up methylene blue in flow 3-5 minutes.
5. A drug washed water.
6. Dry up on air or filtering paper.

Bleaching acid leads to loss dye acid-stable microbes, and they stained in blue Colour. Acid resistant microbes remain red.

3. Painting method on Burri Guinsu

1. mixed drop culture capsule bacteria With drop carcasses on end subject glass. Then cook smear like usually his cook from drops blood.
2. Smear dry up on air and fix in flame burners.
3. For detection bacteria smear stain water fuchsin.

At this way coloring bacteria stained in red Colour, a unpainted capsules stand out in contrast How bezel on black and brown background around bacteria.

4. Way coloring on method Neisser

1. On fixed smear apply blue Neisser 2-3 min.
2. Not rinsing with water inflict solution Lugol 10-30 sec.
3. Smear washed water.

4. Finishing up solution vesuvina — 1 min.

In the culture of yeast-like fungi, there are many grains of volutin. They represent connections, having, in difference from cytoplasm, alkaline reaction and because stained in dark blue Colour. Cytoplasm cells, possessing sour reaction perceives alkaline dye vesuvine and stained in yellow Colour.

5. Way coloring on method Ozheshko

1. On unfixed smear inflict 0.5% solution HCl and warm up on flame burners in flow 2 min. Before appearance vapors.

2. A drug washed water, dry up and fix.

3. Finishing up on method Tsilya-Nelsen.

controversy bacteria after given coloring acquire red Colour, a body bacteria-blue.

TEST TASKS FOR CHECKS KNOWLEDGE

1. If we conditionally choose three main functional and structural components bacteria, then this is will be:

- a) nucleus, cytoplasm, shell;
- b) DNA, cytoplasmic membrane, inclusions;
- c) cell wall, cytoplasmic membrane, nucleus;
- d) shell, cytoplasm, DNA;
- e) ribosome, cytoplasm, nucleus.

2. AT difference from eukaryotic cells bacteria have:

- a) haploid set of chromosomes;
- b) diploid set of chromosomes;
- c) cellular center;
- d) histone proteins.

3. Structurally, the cytoplasmic membrane of bacteria differs from the membranes of other alive creatures topics what:

- a) is three-layer;
- b) c her composition included cholesterol;
- c) capable form endoplasmic network;
- d) able to form mesosome;
- e) capable form spindle division.

4. Rigidity structures bacterial cells provided:

- a) capsule;
- b) cellular wall;
- c) cytoplasmic membrane;
- d) flagella;

e) saws.

5. The form bacteria determined by the structure her:

- a) pili;
- b) cytoplasmic membrane;
- c) cellular wall;
- d) all three components;
- e) unknown to science.

Practical occupation No. 3

Theme: Influence physical factors environmental environments on vitality microbes.

Educational goal:

1. Explore methods sterilization (physical, mechanical, chemical).
2. Learn methods control efficiency sterilization.

The student must know:

1. Methods sterilization
2. Mechanism actions sterilizing factors on molecular structure microorganisms.
3. Concepts contamination and decontamination, disinfection and sterilization, asepsis and antiseptics.
4. Modern sterilization technologies and equipment.
5. Ways control efficiency sterilization and disinfection
6. Control quality sterilization.

The student must be able to:

1. Estimate efficiency sterilization and disinfection.

Plan lessons:

1. Methods sterilization: physical, chemical, biological, mechanical.
2. Device and application ovens Pasteur autoclave, apparatus Koch.
3. Sterilization various medicinal funds in dependencies from them nature, forms, lability to physical factor.
4. Control quality sterilization.
5. concept about asepsis, antiseptic and disinfection.
6. Antiseptics and disinfectants.
7. Principles control quality disinfection.
8. Demonstration antiseptic and disinfectants funds.

Independent Job students:

1. Spend and take into account the results of the experiment to determine the effect of high temperature (80°C) to spore-forming (anthracoid) and asporogenic (E. coli and staphylococcus aureus) microorganisms.

- Fill protocol on form:

Accounting growth culture	Staphylococcus aureus	intestinal wand	Anthracoid
before warming up			
after warming up			

Vegetative forms of pathogenic microorganisms die at 50-60 °C for 30 minutes, and at a temperature of 70 °C for 5-10 minutes. Bacterial spores are more sustainability to high temperatures what explained content in them water in related state, big content salts calcium, lipids and density, layering shells. Consequently, staphylococcus aureus and intestinal wand after warming up are dying a disputes anthracoid survive. it and necessary take account of in evaluation results sowing.

- Fill on one's own table:

No.	Way sterilization	Apparatus	Reliability	sterilizable material
1.	Sterilization in flames			
2.	Plasma Sterilization			

3.	Dry heat			
4	Ferry under pressure			
5	flowing ferry			
6.	Tyndalization			
7.	Filtration			
8.	Physical factors(UFL, gamma rays, ultrasound)			
9	Gas sterilization			
10.	Pasteurization			

INFORMATIONAL MATERIAL ON THEME LESSONSSTERILIZATION

Sterilization is a defecation t. e. complete release of environmental objects environments from microorganisms and them dispute.

Sterilization produce various ways:

1. Physical (exposure to high temperature, UV rays, high pressure, pair, gamma rays, ultrasound).

2. Chemical (usage various disinfectants, antiseptics).

3. biological (application antibiotics).

4. Mechanical (filtration).

AT laboratory practice usually apply physical ways sterilization.

The possibility and feasibility of using one or another method of sterilization conditioned features material, subject sterilization, his physical and chemical properties.

To *physical* ways sterilization can attributed calcination in flame, sterilization dry heat in ovens Pasteur boiling, sterilization fluid ferry in apparatus Koch, ferry under pressure in autoclave, tindalization, pasteurization sterilization UFL, ultrasound.

Mechanical sterilization is carried out by filtration using bacterial filters made from various finely porous materials, the pores of the filters should to be enough small to ensure mechanical delay bacteria. This method sterilize nutritious environment, containing protein, serum, antibiotics; separate bacteria from viruses, phages, exotoxins.

In microbiological practice, Seitz asbestos filters, membrane filters (candles) Chamberlain and Berkefeld.

a) *Seitz filters* - discs made from a mixture of asbestos with cellulose, their thickness is 3-5 mm, diameter 35-140mm;

b) *membrane* filters - made of nitrocellulose, 0.1 mm thick and 35 mm in diameter. AT dependencies from size since designate 1,2,3,4,5;

c) *candles Chamberlain and Berkefeld* — hollow cylinders, closed With one the endcook them from kaolin with admixture of sand and quartz.

Chemical ways sterilization apply limited but they serve for warnings bacterial pollution nutritional Wednesdays and immunobiological drugs (vaccines and sera).

biological sterilization founded on application antibiotics, sometimes phages.

Disinfection - the use of chemicals (phenol, lysol, chloramine, peroxide hydrogen, sublimate, alcohol, etc.) to kill pathogenic bacteria in waste pathological material.

Systematization appliances, processes processing and funds for disinfection andsterilization

Classification tools	Main types tools	Character processing and kinds
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		impacts
critical - penetrate in sterile fabrics or vessels	All invasive surgical tools that have contact with blood-supplied fabrics, scalpels, needles, syringes, implant-yous, burs, root needles, excavators, probes, trowels.	Sterilization - virucidal, sporicidal, tuberculocidal, bactericidal impact. long exposure: gamma-rays, plasma, continuous gas and chemical sterilization, autoclaving (2 atm. 15 min), dry heat (Maxim. mode, 2 hours)
Semi -critical come into contact with mucous shells (per except for some dental tools, listed higher)	- flexible endoscopes, catheters, instruments similar to flexible endoscopes, mirrors,	High level disinfection - virucidal, sporicidal, tuberculocidal, bactericidal impact.

	crowns, tips turbines, a same prints(casts) teeth.	short-term exposure: gamma rays, plasma, short-term gas and chemical sterilization, autoclaving (1-1.5 atm. 15 min), dry heC.
	Thermometers for measuring temperature mucous shells, baths for hydrotherapy.	Medium level disinfection: virucidal, tuberculocidal, bactericidal impact.
	Ultrasonic baths and UV lamps dentists physiotherapy tools, spoons casts.	Means for chemical disinfection with indication of tuberculocidal labeling activity.
Non-critical come into contact with intact skin	Thermometers for measuring temperature skin covers, stethoscopes, cuffs devices for measurements pressure, desktop appliances and t. P.	Disinfection level: bactericidal effects. Funds for chemical disinfection without instructions on presence marking tuberculocidal activity.

TEST TASKS

Specify correct answers:

1. What such sterilization?
 - a) complete desolvation objects from all of us species microbes and them dispute
 - b) destruction pathogenic microorganisms
 - c) destruction of vegetative forms of microorganisms
 - d) prevention hits microorganisms in wound
 - e) destruction on objects specific species microbes
2. What factors are used in autoclaving?
 - a) temperature
 - b) filters
 - c) steam
 - d) pressure
3. What factors are used in the Pasteur oven?
 - a) pressure
 - b) steam
 - c) dry heat
 - d) antibiotics
4. To physical methods sterilization relate:
 - a) ultrasound
 - b) ultraviolet rays
 - c) antibiotics
 - d) filtering
 - e) steam sterilization
 - e) dry heat sterilization
5. List ways sterilization, liberating an object from spore forms microbes:
6. a) irradiation ultraviolet
- b) autoclaving

- c) pasteurization
- d) dry heat
- e) gamma irradiation

Practical occupation No. 4.

Theme: Physiology microbes. Bacteriological method research.

Educational goal:

1. master bacteriological method diagnostics infectious diseases.
2. Explore types nutrition bacteria, principles cultivation microorganisms, classification nutritional avg.
3. Explore methodology receiving pure cultures of bacteria from researched material.

Student should know:

1. Principles cultivation microorganisms.
2. Bacteriological method diagnostics infectious diseases.
3. Nutrients environment, requirements, presented to nutritious Wednesdays
4. Classification nutritional environments, composition and cooking.

Student should be able to:

1. Run the first extraction stage clean culture aerobic bacteria.

Plan lessons:

1. Nutrition bacteria: types, mechanisms receipts nutritional substances in microbial cell.
2. Principles cultivation microorganisms.
3. Bacteriological method diagnostics infectious diseases.
4. Nutrients environments: requirements, presented to nutritious Wednesdays; classification, composition, cooking.
5. Demonstration nutritional avg.
6. Inoculation of the test material (suspensions of microorganisms) on MPA by the method Drygalsky (1 stage).

Independent Job students:

1. Sowing test material according to the method Drygalsky.
2. Familiarization With cooking nutritional avg.

INFORMATIONAL MATERIL ON THEME

Microbiological study held With goal allocation pure cultures microorganisms, cultivation and study them properties. It necessary at diagnosis of infectious diseases, to determine the species of microbes, in research work, for receiving products vital activity microbes (toxins, antibiotics, vaccines and t. P.). For cultivation microorganisms in artificial conditions necessary special substrates — nutritious environment. They are are basis microbiological work and define results Total research. environments must create optimal terms for vital activity microbes.

REQUIREMENTS, PRESENT To WEDNESDAY:

1. Must be nutritious, i.e. contain in an easily digestible form all the substances necessary for satisfaction food and energy needs microorganisms.
2. Have optimal concentration hydrogen ions.
3. To be isotonic for microbial cells.
4. To be sterile.
5. To be wet.
6. Have certain redox potential.
7. To be on opportunities unified.

Need in nutritional substances and properties environments at different species microorganisms are not the same. This eliminates the possibility of creating a universal environment. Besides, on choice toy or different environments affect goals research.

Group classification	Class	Examples
By composition	Simple	<i>Liquid — MPB, peptone water Dense — MPA</i>
	Complex	<i>Liquid - sugar broth Dense -sugar agar, blood agar</i>
By origin	natural	<i>Milk, folded serum, slice raw potatoes</i>
	artificial	<i>Milky salt agar Whey agar Ascites agar Blood agar</i>
	Synthetic	<i>Wednesday Needle, Wednesday 199</i>
By appointment	selective (elective) -for staphylococcus: - for gram (-) cocci and diphtheroids: -for enterobacteria: - for cholera vibrio: -for lactobacilli and mushrooms Differential-diagnostic Universal environments enrichment Preservative	<i>Milk-salt agar, bile-salt agar Serum media Salt media tellurium Media with bile salts Peptone broth and alkaline agar tomato agar, rice agar, agar Saburo</i> <i>Endo, Ploskireva, Levin, Ressel, Giss MPB, MPA, blood agar Wednesday Muller environments With glycerin</i>
By consistency	Liquid semi-liquid Dense	<i>MPB, peptone water, sugar MPB MPJe, gelatinous MPA, blood agar</i>

TEST TASKS

Specify correct answers:

1. What kind nutritious environments are simple?
2. a) Wednesday Endo;
b) MPA;
c) blood agar;
d) MPB;
e) peptone water.
3. Density nutritional Wednesdays depends from content in them:
a) sodium chloride;
b) peptone;
c) agar-agar;
d) sucrose;
e) serum blood.
4. On 1 stage bacteriological method research are solved the following tasks:
5. a) identification clean culture microbes;
b) definition sensitivity to antibiotics;
c) getting isolated colonies;
d) determination of the type of microbe;
e) receiving clean culture.
6. Preferential growth of some species microbes with simultaneous suppression others can receive next types nutritional Wednesdays:
a) selective (elective);
b) simple;
c) complex;
d) preservative;
e) differential diagnostic;
f) universal.
7. AT concept "cultural properties" microbe includes:
a) the nature of growth on nutritional environments;
b) macroscopic characteristic colonies;
c) morphology of microbial cells under microscopy;
d) fermentation carbohydrates on Wednesdays Hiss;
e) Colour pigment colonies or culture;
f) attitude pathogen to coloration on Gram.

Practical occupation No. 5.

Theme: Physiology microbes. Selection pure cultures aerobes and anaerobes..

Educational goal:

1. master methods allocation pure cultures aerobes.
2. Explore types bacteria respiration, ways creation conditions anaerobiosis.
3. master methods allocation pure cultures anaerobes.

The student must know:

1. Principles cultivation microorganisms.
2. Bacteriological method diagnostics infectious diseases.
3. Nutritious environments for cultivation anaerobes

1. Run second stage allocation clean culture aerobic bacteria.

1. Types breathing bacteria.
2. Ways creation conditions anaerobiosis.
3. Methods allocation clean aerobic cultures and anaerobes.
4. Sowing soil talkers for Wednesday Kitta Tarozzi.
5. Nutrients environments for anaerobes, methods cultivation and selection cleanculture anaerobes.
6. Studying cultural properties bacteria.
7. Studying colonies, grown on cups, sown Drygalsky's method.
8. Sowing microorganisms from studied colonies on oblique agar for receivingclean culture (2 stage).
9. Demonstration pigment formation bacteria.
10. Demonstration character growth bacteria on dense and liquid nutritional environments.

1. Completion 1st stage bacteriological method. Studying cultural properties bacteria.
2. From grown colonies on MPA cook smear, paint on Gram.
3. Sowing from researched isolated colonies on oblique agar for accumulation clean culture.
4. Demonstration of anaerobic culture techniques and media for anaerobes: high column of agar, Kitt-Tarozzi medium, thioglycol, Stuart. Demonstration microaerostC. Ways: Fortner, Weinberg.

Breath bacteria. Classification bacteria on type breathing.

The essence of respiration in microorganisms is the receipt of energy generated in the process direct biological oxidation substances oxygen or through dehydrogenation substrate. The accumulation of energy occurs in special structures of bacteria called mesosomes.

1. obligate (strict) aerobes- microorganisms, which are growing and multiply only in presence oxygen. For example: *Vibrio cholerae* *Pseudomonas aeriqinoza*.

2. Obligate anaerobes are microorganisms that grow and reproduce only without oxygen access. For example: *Clostridium botulinum*, *Clostridium tetani*.

3. Facultative anaerobes are microorganisms that can grow and multiply both in the presence of oxygen and in anoxic conditions. For example: *Escherichia coli*, *Salmonella typhi*.

4. Microaerophilic bacteria - microorganisms that grow and reproduce better with a high content of CO₂ and a low content of oxygen. For example: *Helicobacter pylori*, *Campylobacter coli*.

Ways to create anaerobic conditions

a) mechanical — removal (pumping out) air from anaerostat With help vacuum suction. Then the anaerostat is filled with a gas mixture, which consists of 80% nitrogen, 10% hydrogen and 10% carbon dioxide gas;

b) chemical - the absorption of oxygen due to chemicals (alkaline solution pyrogallol, bicarbonate soda);

c) biological (method Fortner) — a joint cultivation anaerobes and aerobes. At the same time, one Petri dish with a dense nutrient medium (more often used Zeissler's medium) are sown with a culture of anaerobes, on the other - a culture of aerobes capable of absorb oxygen vigorously. The miraculous stick culture is used as aerobes (*Serratia marcescens*). The edges cups petri paraffin;

D) physical and chemical — sowing researched material on special environments for anaerobes, for example environments Kitt-Tarozzi and Wilson-Blair (iron sulfite agar). environments front sowing regenerate (boil on water bath in flow 15 minutes) for removal oxygen.

Composition environments Kitta-Tarozzi:

-pieces liver — for adsorption oxygen;

-1% glucose — for implementation anaerobic glycolysis;

- semi-liquid agar — not admits oxygen in thickness environment.

Receipt pure culture anaerobes

1. Method Weinberg (method dilution)

To obtain isolated colonies of anaerobes from the Kitt-Tarozzi medium with growth anaerobic bacteria take away culture Pasteur pipette With soldered end and sequentially lower this pipette first into 3 test tubes with saline, a then — 3 test tubes With melted semi-liquid sugar MPA. After temperature control at 37 °C in recent observed height isolated colonies anaerobes.

2. Method Peretz.

One of the last Weinberg dilutions in semi-liquid agar is poured into the lid cups petri and close her bottom So, to delete air. The edges cups petri paraffin. Sowing researched material on Wednesday Zeissler sectors With subsequent cultivation in anaerosta.

TEST TASKS FOR CHECKS KNOWLEDGE

Specify Everybody correct answers:

1. For cultivation anaerobes without anaerostat used Wednesday:

- a) blood agar;
- b) yolk-salt agar;
- c) Endo;
- d) Kitta-Tarozzi;
- e) Clauberg.

2. At anaerobic type breathing at bacteria absent group enzymes:

- a) dehydrogenases;
- b) flavoproteins;
- c) cytochrome oxidases;
- d) decitinasases;
- e) nucleases.

3. The final electron acceptor in aerobic respiration in bacteria is:

- a) molecular oxygen;
- b) inorganic compounds;
- c) organic connections;
- d) simultaneously organic and inorganic connections;

f) mitochondrial proteins.

4. Wednesday thioglycolic serves for highlights:

- a) obligate aerobes;
- b) obligate anaerobes;
- c) facultative aerobes;
- d) optional anaerobes;

5. Wednesday Kitta-Tarozzi serves for highlights:

- a) obligate aerobes;
- b) obligate anaerobes;
- c) facultative aerobes;
- d) optional anaerobes;

Practical occupation No. 6.

Theme: Physiology microbes. Principles cultivation and identification microbes.

Learning goal: To master the bacteriological method for diagnosing infectious diseases.

The student must know:

- 1. Stages bacteriological method diagnostics.
- 2. Identification clean culture on morphological, tinctorial, cultural and biochemical properties.
- 3. Nutrients environments for study enzymatic activity.
- 4. bacteriophages and them application.
- 5. classification enzymes.

Student should be able to:

- 1. Do sowing researched material on environments gissa and MPB.
- 2. Define antibiotic sensitivity methods standard disks.
- 3. Verify purity dedicated culture.

Plan lessons:

- 1. Enzymes, classification. Enzymes that break down carbohydrates, proteins, fats, pathogenicity enzymes. Culture media used for enzymatic activities microorganisms.
- 2. Examination purity dedicated culture macro and microscopically.
- 3. Identification of a pure culture of bacteria by morphological, tinctorial, cultural, biochemical, phagolyzable properties (3 stage).
- 4. bacteriophages, them application for identification bacteria.
- 5. Definitions antibiotic sensitivity method standard disks.

Independent Job

- 1. Cooking smear, coloring on Gram.
- 2. Sowing culture on Hiss environments and MPB.
- 3. Definition antibiotic sensitivity.
- 4. Demonstration nutritional Wednesdays for study enzymatic activity microorganisms.
- 5. Demonstration phenomenon bacteriophage on dense and liquid nutritional environments.

6. Demonstration allocation clean culture mobile microorganisms on method Shukevich.

INFORMATIONAL MATERIAL ON THEME

III stage of the study. After 24 hours, a homogeneous growth was found on the beveled MPA. in the form of a continuous yellowish coating. In order to check the purity of culture isolation a smear is prepared from the test tube, stained according to Gram and microscopied. For rate purity culture necessary browse not less 10 fields vision. Finding insmears co oblique MPA only grape-like located gram-positive cocci, testifies about purity allocated cultures.

For definitions biochemical bacteria produce sowing allocated culture in color series media (glucose, lactose, mannitol, sucrose, maltose and in the MPB for determining indole and hydrogen sulfide), or determine these properties using the Indicator System Paper disks (NIB).

crops placed in thermostat at temperature 37°C on 24 hours.

enzymes bacteria

Enzymes are highly specific biological catalysts, without which life and reproduction are impossible. A large number of reactions that occur during life bacterial cell, indicates the existence in bacteria of a significant amount enzymes. Enzymes — substances protein nature With big molecular weight. Some from them relate to proteins other are complex proteins. They are are built from two protein parts and a non-protein part called the prosthetic group. AT it may contain vitamins. nucleotides, iron atoms, etc. The relationship between the protein part enzyme and th prosthetic group maybe to be durable and fragile. At in the presence of a weak bond in solutions, dissociation of the enzyme occurs and, in this case, be released free prosthetic group.

Easy dissociating irostetic groups enzymes called coenzymes. Usually enzymes subdivided for the next main groups:

1. Oxidoreductases. Everybody enzymes, catalytic oxidizing restorative reactions.
2. Transferases. catalytic transfer those or other groups (for example amino groups, phosphate leftovers and t. d.
3. Hydrolases that cleave Gt or other compounds by hydrolysis; to this class relate same phosphatase and deampnase — enzymes, chipping off respectively hydrolytic through phosphate or ammonium groups from various organic connections.
4. Lyases, enzymes that cleave certain substances from substrates in a non-hydrolytic way groups (for example, CO₂, NgO, SH₂ and t. d.).
5. isomerases, catalytic intramolecular perestroika in substrate.
6. Ligases (synthetases) - a class of enzymes that catalyze the attachment to each other two molecules with simultaneous breaking of the pprophosphate bond in triphosphates (for example, generators C — O, FROM - N or FROM — S connections).

Saprophytes have the highest enzymatic activity; less this property is expressed in pathogenic bacteria. Study of enzymes of pathogenic bacteria is of exceptional importance, since on the basis of the determination of the enzymatic activity of microbes, it is possible to differentiate different types and determine the nature of that or other pathogen. Along with this, the enzymatic activity of microbes defines pathogenesis and clinical picture infectious diseases.

Enzymes differentiate on exo and endoenzymes. Exoenzymes stand out cell in external Wednesday, carry out processes splitting macromolecular organic connections to simpler available for assimilation.

Enzymes bacteria subdivided on constitutive and inducible. To first group relate those enzymes, which synthesized bacterial cell outside depending on Togo, on which environment bacterium is grown. inducible enzymes produced given bacterium only in answer on action specific inductor, present in environment.

TEST TASKS FOR CHECKS KNOWLEDGE

Specify Everybody correct answers:

1. Saccharolytic properties microbes study on environments:
 - a) MPL, MPB;
 - b) Hiss;
 - c) Ressel;
 - d) bloody agar;
 - e) in the test system API;
 - f) in test system Lachema.
2. Biochemical activity microbes on dense environments gissa taken into account on:
 - a) color change environment;
 - b) education draft; in) gas formation;
 - D) break environment.
3. Proteolytic enzymes microbes are being studied on environments:
 - a) with carbohydrates;
 - b) MPB;
 - c) milk;
 - d) gelatin
4. Enzymes in chemical relation contain:
 - a) substrate;
 - b) apoenzyme; c) coenzyme;
 - d) metabolite
5. Main goals applications differential diagnostic Wednesdays:
 - a) study of biochemical activity microbes;
 - b) study cultural properties microbes;
 - c) definitions sensitivity to antibiotics;
 - d) differentiation various species microbes;
 - e) transportation material in laboratory.

Practical occupation #7

Delivery of the module on the topic "Influence on microbes of physical and chemical factors. Physiology microbes. Principles cultivation and identification microbes."

Educational goal:

1. Explore methodology allocation pure cultures bacteria from researched material.

The student must know:

1. Methods allocation pure cultures bacteria.

The student must be able to:

1. Make conclusion on allocation clean culture microorganism.

Plan lessons:

1. Selection clean culture aerobic and anaerobic bacteria (conclusion).
2. Change module.

Independent work of students: IV

stage research. Accounting biochemical properties.

glucose	lactose	mannitol	sucrose	maltose	indole	hydrogen sulfide
To	To	To	To	To	+	-

- to — acid

CONCLUSION: from mixtures bacteria highlighted and identified *Staphelococcus* spp. on basis morphological, tinctorial (gram positive, grape-shaped located cocci) and cultural (smooth convex colonies of golden color) and biochemical properties.

Practical occupation No. 8.

Theme: General virology. Methods virology. Bacteriophages and phage typing.

Educational goal:

1. Explore morphology and ultrastructure viruses.
2. Explore structure and morphology bacteriophages.

The student must know:

1. morphology, ultrastructure classification viruses.
2. morphology, ultrastructure classification bacteriophages.

The student must be able to:

1. Find viral inclusion method light microscopy.
2. Find viral inclusion method luminescent microscopy.

Plan lessons:

1. Peculiarities biology viruses .
2. Principles classification viruses.
3. Types interactions viruses With cell.
4. Morphology and structure bacteriophages, them practical application in medicine.
5. Change module.

Independent Job students:

- Studying demonstrations phenomenon bacteriophage on dense and liquid nutritional environments.
- Studying demonstrations intracellular inclusions (body Babesha-Negri).

INFORMATIONAL MATERIAL ON THEME

VIRUSES

Viruses possess properties, not allowing apply for them study ordinary methods microbiological research.

Distinctive properties viruses:

1. smallest sizes, measurable thousandths shares micron - millimicrons -from 8-10 m before 300-400 m.
2. Filterability through special finely porous filters, not passing other microorganisms.
3. non-cellular structure.
4. Absolute parasitism, those. ability live and multiply only in alive cells.

The form viral particles It has some types:

1. rod-shaped
2. spherical (spherical)
3. Cuboid
4. Capitate (spermatozoa)
5. Filiform

Mature viral particles, called *virions*, have the following structure: the central part is a DNA or RNA molecule, which forms a *nucleoid*. Around located protective protein shell, called *capsid*, built from morphological units, called *capsomeres*. Some complex virions have external shell, called *supercapsid*.

For microbiological diagnostics viral infections in the present time apply three major methodical approach:

1. Virological diagnosis - based on isolation from the test material virus and his subsequent identification.

2. Serological diagnostics - definition specific immunological changes in the body under the influence of viruses (more often Total With help diagnosticums reveal in blood serum antiviral antibodies).

3. Molecular biological diagnostics - detection in clinical material nucleic acid fragments of pathogenic viruses using probes (hybridization NK) or PCR.

Individual viruses larger than 200 m can be stained according to Romanovsky - Giemsa; smaller viruses (variola viruses) can only be detected using special ways processing.

Bacteriophages differ on chemical structure, type nucleic acid, morphology and nature of interaction with bacteria. The size of bacterial viruses hundreds and thousands once smaller microbial cells.

Typical phage particle (virion) includes from heads and tail. Length tail usually in 2 - four times more diameter heads. AT head contained genetic material - single stranded or double stranded RNA or DNA With enzyme transcriptase in inactive state, surrounded by protein or lipoprotein shell - **capsid**, preserving genome outside cells.

Nucleic acid and capsid together make up the nucleocapsid. Bacteriophages can have icosahedral capsid, assembled from sets copies one or two specific proteins. Usually angles are made up of pentamers squirrel, and the support of each side of hexamers of the same or similar protein. Moreover, phages can be spherical in shape, lemon-shaped or pleomorphic. Tail is yourself protein handset - continuation protein shells heads, in basis tail available ATPase, which regenerates energy for injections genetic material. Exist same bacteriophages With short offshoot, not having process and filiform.

Phages, like all viruses are absolute intracellular parasites. Although they transfer all the information to start their own reproduction in the appropriate host, they lack the mechanisms to energy production and ribosomes for synthesis squirrel. Some phages have several thousand bases in their genome, while the G phage, most large from sequenced phages, contains 480 000 steam grounds

twice

more than the average for bacteria, although still not enough genes for the most important bacterial organelles like ribosomes.

A large number of isolated and studied bacteriophages determines the need their systematization. Classification viruses bacteria has undergone changes: based on characterization host virus, taken into account serological, morphological properties, a then structure and physical and chemical composition virion .

Currently, according to the International Classification and Nomenclature of Viruses bacteriophages, in depending on nucleic type acids share on DNA- and RNA- containing.

Based on their morphological characteristics, DNA-containing phages are classified into the following groups: families: myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Plasmaviridae, Corticoviridae, Fuselloviridae, tectiviridae, microviridae, Inoviridae Plectovirus and Inoviridae Inovirus.

RNA containing: cystoviridae, Leviviridae

By character interactions bacteriophage With bacterial cell distinguish virulent and moderate phages. Virulent phages may only increase in quantity through lytic cycle. Process interactions virulent bacteriophage with cell consists of several stages: adsorption bacteriophage on cage, penetration in cell, biosynthesis components phage and then assembly, exit bacteriophages from cells.

Initially bacteriophages attached to phage-specific receptors on surface of the bacterial cell. The tail of the phage with the help of enzymes located on it end (in mostly lysozyme), locally dissolves shell cells, shrinking and contained in head DNA injected in cell, at this protein shell bacteriophage remains outside. injected DNA causes complete perestroika metabolism cells: stops synthesis bacterial DNA, RNA and proteins. DNA bacteriophage starts be transcribed With help own enzyme transcriptase, which after hits in bacterial cage is activated. First, early and then late mRNAs are synthesized, which enter the ribosomes. host cells, where early (DNA polymerases, nucleases) and late (proteins) are synthesized capsid and tail process, enzymes lysozyme, ATPase and transcriptase) squirrels bacteriophage. replication DNA bacteriophage going on on semi-conservative mechanism and carried out With participation own DNA polymerases. After synthesis late proteins and completion replication DNA comes final process - maturation of phage particles or fusion of phage DNA with an envelope protein and formation mature infectious phage particles.

Duration this process maybe make up from several minutes before several hours. Then going on lysis cells, and released new mature bacteriophages. Sometimes the phage initiates a lysis cycle, which leads to cell lysis and release of new phages. Alternatively, the phage can initiate lysogenic cycle, at which he instead of replication reversible interacts With genetic system host cell, integrating in chromosome or remaining in form plasmids. So the way viral genome replicated synchronously With DNA host and division cells, and such a state of the phage is called prophage. Bacterium, containing prophage, becomes lysogenic as long as, under certain conditions or spontaneously prophage not will be stimulated on implementation lysing cycle replication. Transition from lysogeny to lysis is called lysogenic induction or prophage induction. For induction phage is strongly influenced by the state of the host cell prior to induction, same How Availability nutritional substances and other terms, having place in moment induction. Poor growth conditions favor the lysogenic pathway, while good terms contribute lysing reactions.

Highly important property bacteriophages is them specificity: bacteriophages lyse cultures of a certain type, moreover, there are so-called typical bacteriophages, lysing options inside kind, although meet polyvalent bacteriophages, which parasitize in bacteria different species .

Viruses highlighted in separate "kingdom" - Viga. They are contain only one type nucleic acids, do not have a cellular structure, do not have independent metabolism substances being intracellular parasites reproduction viruses carried out disunited way.

According to the international classification, all viruses are subdivided according to the type of nucleic acids on 2 subtype - RNA- and containing DNA. Further separation viruses is carried out on the basis of the size of the viruses, the type of symmetry in the formation of capsids, availability or absence external shells and quantity contained in them capsomeres.

VIROLOGICAL METHOD RESEARCH is main and most authentic, allows highlight virus

from researched material With subsequent hisidentification. In order to accumulate virus-containing material, chickenembryos and tissue cultures (artificially cultured cells of a particular tissue). Tissue cultures are maintained on natural (medium 27, Enders) and synthetic (Wednesday 199, Needle, Melnik-Riordan) nutritional environments cooked on basis solutions Hanks and Earl. cultivated they in regular test tubes cups of carrel, test tubes Barsky.

Methodology infections chicken embryo

There are several ways to infect a chicken embryo. Most often the material injected into the allantoic and amniotic cavities, onto the chorionallantoic membrane and into yolk sac. Before infection, the egg shell above the air chamber is processed 70% alcohol, burned on a flame, smeared with 2% iodine tincture, rubbed again alcohol and burn.

When infected in the allantoic cavity in the shell above the air chamber (borders which is outlined in pencil in advance when translucent eggs in an ovoscope) do a small hole with scissors or a scalpel. tuberculin syringe injected 0.1-0.2 ml virus-containing material on depth 2-3 mm below borders air cameras. Puncture in shell poured molten paraffin. Opening infected embryos are produced at the time of maximum accumulation of the virus (after 48-72 hours of incubation at temperature 37 FROM) after processing shells alcohol and 2%

with a solution of iodine, it is dissected and discarded, the shell membrane is carefully removed and consider the chorionallantoic membrane around the site of infection for the presence of foci defeats (hemorrhage, whitish foci defeats).

Classification cellular crops:

- **primary** receive directly from fabrics animal and human through destruction proteolytic enzymes (trypsin, collagenase) intercellular substances. Dissociated cells placed in a nutrient medium are able to attach to the surface of the culture vessel and multiply, forming a monolayer - a layer with a thickness of one cell. With the help of special reagents, cells can be removed from the surface of one vessel and transplant in another. Such manipulation called **passage**. Primary culture withstand not more 5-10 passages.

- **transplanted** (passage) cellular culture able withstand unlimited number of passages. They originate from tumor cells that have lost differentiation and not having restrictions growth.

- **semi-transplantable** (diploid) cultures are fibroblast-like cells that capable of rapid reproduction, withstand up to 30-60 passages and retain the originalkit chromosomes.

Viruses can reproduce only in the cells of a living organism. Due to this viruses are cultivated by infecting chick embryos or tissue cultures, and suckling animals.

Detection (indication) viruses Virus

detection in chick embryo1. Death

2. The appearance of an odor upon

opening 3. Cloudiness liquids in cavities

four. Education sores and hemorrhages on shells

Biological method research is in contagion sensitive to virus animal researched material, studying clinical and pathoanatomical paintings diseases. AT framework this method are used various animals: monkeys, rabbits, guinea pigs, dogs, mice, rats. Ways infections: subdural, intracerebral, intranasal and other.

Ways detection virus in body laboratory animals differ in dependencies from kind animal and type virus.

Detection viruses in culture cells

Revealing on cytopathic action (CPD). JPC is yourself degenerative changes in cells that appear as a result of reproduction in them viruses.

Distinguish complete and partial degeneration cells monolayer.

With complete degeneration caused, for example, by polio viruses, Coxsackie and ECHO, cells of the monolayer undergo significant changes, more of them slough off co glass. Remaining single cells wrinkled

Partial degeneration has several varieties: 1 .By type cluster formation (adenoviruses);

2. By type focal destruction (smallpox, flu);
3. According to type syncytium formation (measles, parotitis, parainfluenza, herpes, HIV).
 - proliferative type changes typical for some oncogenic viruses, transforming cells in malignant.
 - Intracellular inclusion* formed at reproductions some viruses in cytoplasm and nucleus of cells (smallpox, rabies, influenza, herpes, etc.) They are found when microscopy after coloring monolayer on Romanovsky - Giemsa, a same at luminescent microscopy.
 - Salk color test.** As a result of the vital activity of cells in a nutrient medium accumulate sour products. At result this Colour incoming in composition environments indicator (phenol red) turns orange. When cell culture is infected cytopathogenic viruses such as enteroviruses or reoviruses, cell metabolism suppressed medium pH and her Colour not are changing (Wednesday remains red).
 - Reaction hemagglutination.** At basis this reactions lies ability viruses, containing hemagglutinin receptors, "glue" erythrocytes. If eat hemagglutinins - RGA+(umbrella), if No - RGA - (button).
 - Reaction hemadsorption.** Mechanism similar With RGA.

TEST TASKS

1. For microbiological diagnostics viral infections apply the following major methodical approach
 - A) bacteriological diagnostics;
 - b) virological diagnostics;
 - c) serological diagnostics;
 - d) molecular biological diagnostics.
2. Viruses multiply only:
 - a) in alive systems;
 - b) on meat-peptone agar;
 - c) on differential diagnostic media;
 - d) on elective environments.
3. First stage virological diagnostics is receiving and preparation
 - a) cultures cells;
 - b) chicken embryos;
 - c) sensitive laboratory animals;
 - d) differential diagnostic avg.
4. Reveal viruses:
 - a) By cytopathic action;
 - b) By plaque formation;
 - c) color sample;
 - d) By biochemical properties.
5. Virus found in chick embryos
 - a) by changing the chorioallantoic membrane;
 - b) RGA (Reaction agglutination);
 - c) RSK (Complement fixation reaction);
 - d) RP (Reaction precipitation).
6. What are bacteriophages?
 - a) bacteria;
 - b) viruses;
 - c) phagocyte cells;
 - d) mushrooms.
7. What kind microbes not have cellular buildings?
 - a) viruses;
 - b) mycoplasmas;
 - c) chlamydia;

- d) mushrooms.
8. What contains difficult organized virus?
- a) two types nucleic acids;
 - b) one type of nucleic acid (either DNA or RNA);
 - c) supercapsid;
 - d) capsid
9. Viruses opened:
- a) L. Pasteur;
 - b) R. Koch;
 - c) I. Ivanovsky;
 - d) A. Mechnikov.
10. Extracellular the form existence viruses
- a) virion;
 - b) capsid;
 - c) capsomere;
 - d) supercapsid;
 - e) elementary bodies.

Practical lesson number nine

Theme: Basics genetics. Molecular biological method diagnostics.

Polymerase chain reaction, her varieties.

Educational goal:

1. Explore ways transmission genetic information between bacteria: transduction, transformation and conjugation.
2. Explore basics biotechnology and genetic engineering.

The student must know:

1. Forms variability microorganisms.
2. Terms occurrence variability microorganisms, their practical meaning.
3. Essence biotechnology, goals and tasks.
4. Microorganisms and processes, applied in medical biotechnology.
5. Application genetic engineering in biotechnology.
6. genetic recombination microorganisms.

Student should be able to:

1. Take into account results experience transformations.
2. Take into account results experience transduction.
3. Take into account results experience conjugation.

Plan lessons:

1. Medical biotechnology.
2. genetic recombination: transduction, conjugation, transformation
3. Role genetic recombinations in genetic engineering and medical biotechnology.
4. The use of plasmids in genetic engineering research.
5. Application genetic and molecular biological methods in diagnostics infectious diseases: PCR, method molecular probes.
6. Biopreparations, obtained by the method genetic engineering (vaccines, monoclonal antibodies, hormones, diagnostics).

Independent Job students

1. Accounting results experience transformations.
2. Accounting results experience transduction.
3. Accounting results experience conjugation.
4. Specify correct answers in test tasks.
5. Sketching tables.

INFORMATIONAL MATERIAL ON THEME LESSONS

staging experience transformation

Recipient — strain *Bacillus subtilis* str (hay wand, sensitive to streptomycin); donor — DNA, dedicated from strain *C. Subtilis* str (sustainable to streptomycin). selective Wednesday for selection recombinants (transformants) nutritious agar, containing one hundred U/ml streptomycin.

To 1 ml of *B. subtilis* broth culture add 1 µg/ml of DNase solution in 0.5 ml solution chloride magnesium for destruction DNA, not penetrated in bacterial cells recipient strain, and withstand in flow five min. For definitions quantities formed streptomycin-resistant recombinants (transformants) 0.1 ml undiluted mixtures sown on selective Wednesday in cup Petri. For definitions quantities cells recipient culture in isotonic solution chloride sodium cook 10x breeding before 10^{-5} - 10^{-6} (for receiving countable quantities colonies), inoculated 0.1 ml on nutrient agar without streptomycin, and for control - on agar co streptomycin. On latest environment recipient culture not must grow, because the she sensitive to streptomycin. Sowing incubate at 37 ° FROM. On next day take into account results experience and define transformation frequency on the ratio of the number of grown recombinant cells to the number of recipient cells strain.

Let us assume that when seeding 0.1 ml of the culture of the recipient strain in a dilution of 10^{-5} has grown 170 colonies, at sowing 0.1 ml undiluted mixtures — 68 colonies recombinant strain. Because the each the colony formed in result breeding only one bacterial cell, then in 0.1 ml sown culture recipient contains 170×10^5 viable cells, and 1 ml - 170×10^6 or 1.7×10^8 . At the same time, 0.1 ml of the mixture contains 68 recombinant cells, and 1 ml contains 680, or 6.8×10^2 .

So way, the frequency transformation in this experience will be is equal to:

$$\frac{6.8 \cdot 10^{-2}}{1.7 \cdot 10^8} = 4.0 \cdot 10^{-6}$$

staging experience specific transduction

Recipient — strain *E. coli lac-*, - deprived 3-galactosidase operon, controlling lactose fermentation. Transducing phage - phage X dgal, in the genome whom part genes replaced (3-galactosidase opera-ron *E. coli*. He is defective, i.e., unable to cause a productive infection ending in lysis coli, and is denoted by the letter d (phage dgal) with the name contained in the genome bacterial operon gal. Selective Wednesday — Wednesday endo, on which lactose-negative bacteria of the recipient strain form colorless colonies, lactose-positive colonies recombinant strain acquire red Colour With metallic tint. K 1 ml 3 hour broth culture recipient strain add 1 ml of transducing phage dgal at a concentration of $10^6 - 10^7$ particles per 1 ml. Mixture incubated for 60 minutes at 37°C , after which a series of 10-fold dilutions is prepared (in dependencies from supposed concentration bacteria) for receiving countable the number of colonies. From a test tube with a dilution of 10^{-6} , 0.1 ml of culture is inoculated per 3 cups petri co environment Endo and evenly distribute liquid spatula on surfaces environment.

crops incubate in flow 1 days, after what note results experience and the frequency of transduction is calculated from the ratio of the number of recombinant cells (trans- ductants), discovered for everyone cups, to number cells recipient strain.

For example, after inoculation of 0.1 ml of the mixed culture at a dilution of 10^{-6} on 3 plates of Endo medium grew 138, 170 and 160 colorless colonies of the recipient, respectively. strain, on the first and last cups - 5 and 1 colonies of red transductants. Consequently, transduction frequency in this case will is equal to:

$$\frac{(5 + 1) \cdot 10 \cdot 10^{-6}}{(138 + 170 + 160) \cdot 10 \cdot 10^{-6}} = \frac{6}{48} = 1.3 \cdot 10^{-2}$$

staging experience conjugations With goal transmission fragment chromosomes, which contains gene leu, controlling synthesis leucine.

Donor - strain *E. coli* K12 Hfr *leu* Str^S; recipient - strain *E. Coli* K12F- - *leu*+ Str^R. hfr — designation states, for whom characteristic high frequency recombination. Selective Wednesday for allocation recombinants -minimum glucose salt Wednesday: KN_2RO_4 — 6.5 G, MgSO_4 — 0.1 G, $(\text{NH}_4)_2\text{SO}_4$ — 1 G, $\text{Ca}(\text{NO}_3)_2$ — 0.001 G, FeSO_4 — 0.0005 G, glucose — 2 G, streptomycin — 200 U/ml distilled water — 1 l.

To 2 ml of a 3-hour culture of the recipient, add 1 ml of the broth culture of the donor. The cultures are incubated at 37°C for 30 minutes. Then the mixture is diluted to $10^{-2} - 10^{-3}$ and sown on 0.1 ml per selective agar Wednesday at cups Petri, on which will grow only colonies recombinants. AT quality control on that same Wednesday sown donor and recipient strains, which not will grow on her, t. to. the first strain sensitive to streptomycin, and the second auxotrophen for leucine. In addition, the culture of the donor strainsown on a selective medium without streptomycin, and the culture of the recipient strain - oncomplete medium (nutrient agar) with antibiotics to determine the number of viable cells. Crops are incubated at 37°C until the next day. After counting the number of grown colonies define frequency recombinations on relation quantities recombinant cells to recipients.

For example, after inoculation of 0.1 ml of a mixture of donor and recipient cultures in dilution 10^{-2} grew 150 colonies of recombinants, and after inoculation of 0.1 ml of the culture of the recipient from dilutions 10^{-6} 75 colonies. So the way frequency recombination will be is equal to:

$$\frac{150 \cdot 10 \cdot 100}{75 \cdot 10 \cdot 10^6} = \frac{1.5 \cdot 10^5}{7.5 \cdot 10^8} = 2.0 \cdot 10^{-4}$$

Polymerase chain reaction (PCR) — one from contemporary molecular genetic methods, founded on principle multiple copying (amplifications) certain site DNA or RNA. AT result this process amount of determined DNA in sample increases in dozens million once, what does possible subsequent detection amplified DNA. So the way PCR allows you to identify negligible fragments of DNA characteristic of a particular species germs, and exactly to identify this view.

PCR in cage was open more thirty years old back Nobel laureates A. Kronberg and D. Ledeborg. Principle of the PCR method invitro was developed by K. Mulis in 1983, same become Nobel laureate. Almost immediately appeared messages about his practical application. However in this period due to absence necessary PCR equipment was carried out by manually transferring the tubes to thermostats with the desired temperature. The enzyme DNA polymerase, necessary for DNA synthesis, was destroyed after everyone denaturation stage (at 95°C), so it needed to be constantly added new servings.

In 1988, a thermostable DNA polymerase was obtained from the bacterium *Thermophilus aquaticus*, living in hot springs. Were developed special appliances for amplifications (thermocyclers). Modern laser sequencing technologies created (deciphering the nucleotide sequences of DNA). This led to PCR becoming accessible for wide applications in laboratory practice.

AT the present time most quickly develop five major directions genetic diagnostics:

- infectious diseases (tuberculosis, gonorrhea, viral infections - hepatitis B and FROM, HIV CMV and etc.),
- oncological diseases,
- genetic diseases,
- identification personalities (transplantation bodies and fabrics, judicial medicine, definition paternity),
- diagnostics pathogens in food.

Test material: blood, serum, lavage masses, sputum, saliva, gastric the juice, biopsy material, strokes, flushes.

Staging PCR includes the following stages:

1. Selection DNA (RNA) from researched material (sample preparation).

Cells lyse detergents or high temperature. Then separated DNA from cell debris and destroy cellular nucleases. Everybody it provide appliances: minicentrifuges, developing speed 12000 - 14000 rpm per minute, vortexes for mixing, mini thermostats for test tubes providing rapid change temperature from +30°C up to +100°C.

2. Immediate amplification allocated plots (copies) nucleic acids.

PCR provides fast and multiple multiplication, amplification (amplification - amplification, increase) in the number of genome fragments. For this, in a test tube with isolated DNA is added with the necessary reagents and placed in an amplifier (thermal cycler). This the device allows you to cyclically change and maintain temperature drops in the test tube for several tens of degrees in a few seconds. If the test tube contains the desired DNA, then her going on a number of processes:

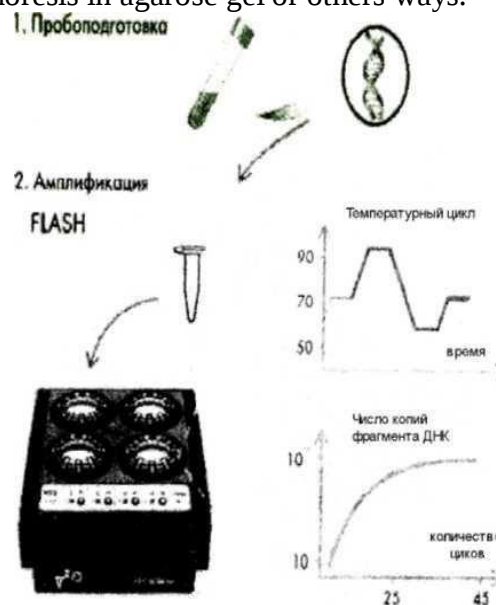
- As a result of heating to 94 -95 ° C, the double strand of DNA is divided into two separate chains.

- To single stranded target DNA joins primer.

Primer — this is sequence from 15 — thirty nucleotides, complementary marker DNA fragment. When creating the optimum temperature (45-70°C), binding (annealinD) of the primer to the corresponding DNA region: one primer on one threads, another — on second threads DNA. Annealing leaks in accordance With rule complementarity of Chargaff, meaning that in a double-stranded DNA molecule, opposite adenine is always thymine, a against guanine — cytosine.

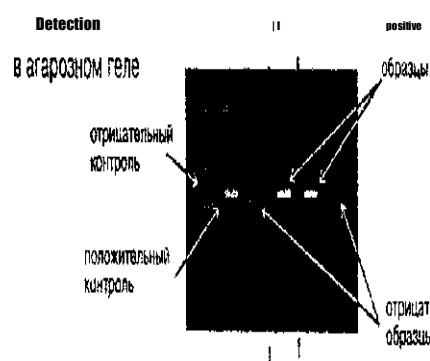
- Synthesis (elongation) - completion of the second chainDNA.

DNA polymerase adds nucleotides to primers, completing double-stranded DNA fragments (~ at 72°C). The newly synthesized DNA fragments serve as a template for synthesis of new chains in the next cycle of amplification - this is the chain reaction in PCR. As a result, the number of fragment copies increases exponentially and after 25 cycles of amplification, 10^6 copies of the fragment are synthesized. After 30 - 40 cycles synthesized such number DNA, which can visually take account of after electrophoresis in agarose gel or others ways.



3. Definition (detection) products PCR, received on second stage.

Revealing accumulated product often carried out at help electrophoresis in 2-



3% agarose gel containing ethidium bromide (a specific fluorescent dye DNA). By absorbing ultraviolet light, the dye associated with DNA fluoresces. As a result, an orange stripe is visible at the level of the control DNA. In addition, they use enzyme hybridization method or real-time PCR using fluorescent dyes.

Test control:

1. What such transformation?
 - a) recovery damaged DNA;
 - b) broadcast genetic information at contact bacterial cells different "sexual" orientation;
 - c) broadcast genetic information With help fragment DNA;
 - d) broadcast genetic information from cells donor cage recipient With help bacteriophage.
2. For conjugations characteristic:
 - a) broadcast genetic material at help bacteriophage;
 - b) needed contact cells donor and recipient;
 - c) broadcast genetic material With help RNA;
 - d) transfer of genetic material with the help of the sex factor.
3. For transduction characteristic:
 - a) broadcast genetic material at help bacteriophage;
 - b) needed contact cells donor and recipient;
 - c) broadcast genetic material With help RNA;
 - d) broadcast genetic material With help gender factor.
4. At what microorganisms material basis of heredity is RNA?
 - a) at bacteria;
 - b) at spirochetes;
 - c) in RNA-containing viruses;
 - d) in DNA-containing viruses;
 - e) at mycoplasmas.
5. What are extrachromosomal genetic structures?
 - a) ribosomes;
 - b) polysomes;
 - c) plasmids;
 - d) mesosomes;
 - e) early zones.

Practical occupation #10

Theme: Antibacterial chemotherapy

Educational goal:

1. Explore mechanism actions antibiotics on microbial cell.
2. To study the method for determining the sensitivity of bacteria to antibiotics.

The student must know:

1. Range actions antibiotics on microbial cell.
2. Definition sensitivity (methods indicator disks and cassette).

The student must be able to:

1. describe results sensitivity clean culture to antibiotics.
2. Define sensitivity bacteria to antibiotics method indicator disks.

Plan lessons:

1. antibiotics, definition, chemical classification structure, spectrum types and mechanism

actions.

2. Chemotherapeutic drugs, mechanism their actions on microbial cell.
3. Mechanisms medicinal sustainability bacteria.
4. Side effects of antibiotics and synthetic antimicrobial drugs.
5. Methods and units measurements antimicrobial activity.
6. Antiviral chemotherapy drugs.
7. Demonstration antibiotics With various mechanisms and spectro actions.
8. Change module.

Independent Job students

1. Take into account results disk antibiograms.
2. Take into account results cassette micromethod.
3. Design protocol research.

INFORMATIONAL MATERIAL ON THEME LESSONS

All antibiotics are selective. Their relative harmlessness for human determined, before Total, topics what they specifically suppress such metabolic processes in microbial cage or virus, which missing in eukaryotic cage or unavailable for them. AT this relation unique is mechanism actions beta-lactam antibiotics. targets for them are transpeptidases that complete the synthesis of cell wall peptidoglycan. Because the cellular wall eat only in prokaryotes, in the eukaryotic cage No targets for beta- lactam antibiotics. Transpeptidase present yourself kit enzyme proteins, localized in the cytoplasmic membrane of the bacterial cell. Separate beta lactams differ in the degree of affinity for a particular enzyme, which received the name of penicillin-binding proteins. Therefore, the biological effect of beta-lactam antibiotics different: bacteriostatic, bactericidal, lytic.

Except beta-lactam antibiotics, synthesis cellular walls amaze such antibiotics like bacitracin, fosfomycin, cycloserine, vancomycin, ristomycin, however otherwise by, how penicillin. Everybody they, Besides cycloserine, cause bactericidal Effect.

Mechanism actions such antibiotics, How chloramphenicol, tetracyclines, streptomycin, aminoglycosides, erythromycin, oleandomycin, spiramycin and other macrolides, lincosamides, fusidic acid, is associated with inhibition of protein synthesis at the level ribosomes 70S. Although bacterial ribosomes 70S have the same structure in principle as ribosomes of 80S eukaryotic cells, their proteins and protein factors involved in the work protein-synthesizing systems, different from such ribosome 80S. This explained selectivity of action specified antibiotics for protein synthesis bacteria.

Different antibiotics differently block synthesis squirrel. Tetracyclines block binding at-RNA on A-section ribosomes 70S. Chloramphenicol suppresses peptidyl transferase reaction. Streptomycins impede transformation initiator complex into a functionally active ribosome. Erythromycin blocks reaction translocations. Puromycin, joining to growing end synthesized polypeptide chains, causes premature department her from ribosomes. Mechanism The action of fluoroquinolones is associated with their selective inhibition of bacterial enzymes. DNA gyrase, participating in replication DNA. Fluoroquinolones contact co specific plots DNA, which created impact DNA gyrase, and suppress her activity.

Rifampicins inhibit the activity of DNA-dependent RNA polymerases, as a result of which bacteria suppressed processes transcription.

Activity antitumor antibiotics connected with the what they or are inhibitor synthesis DNA (bruneomycin), or suppress activity DNA dependent

RNA polymerase, t. e. blocks transcription (anthracyclines, actinomycins, olivomycin).

Accounting for the results of determining the sensitivity isolated from the test material microorganisms to antibiotics held next way: on working table there is a Petri dish on which the isolated from the studied material was sown microbe and were inflicted on equal to distance friend from friend discs With antibiotics (this method work outlined in practical guide).

The student needs draw a conclusion about the degree of sensitivity of the isolated culture to antibiotics. Meaning given research comes down to next: surface nutritional environments on cup moisten suspension dedicated clean culture in physical solution and so way achieved uniform distribution culture on all cup.

Disks with antibiotics are placed “on top” of the inoculation and the plates are incubated in a thermostat. From disks, impregnated every separate antibiotic going on diffusion antibiotics in the thickness of the agar. The more sensitive a culture is to an antibiotic, the less efficiency of concentration and the larger the diameter of the zone of growth inhibition of culture around certain disk. At this result accounted for following scheme (table).

culture highly sensitive	diameter zones growth inhibition bacteria thirty and more mm.
culture medium sensitive	diameter zones oppression growth bacteria not less 20 mm.
culture weakly sensitive	diameter zones oppression growth bacteria not more 10 mm.

test control

- Synthesis cellular walls suppress antibiotics:
 - polymyxin
 - aminoglycosides
 - cephalosporins
 - tetracyclines
- Violation of the function of the cytoplasmic membrane is noted under the action of:
 - cephalosporin
 - macrolides
 - levomecithind
 - nystatin
- Antibiotics, inhibitory synthesis squirrel on ribosomes bacterial cells:
 - penicillin
 - polymyxin
 - aminoglycosides
 - amphotericin AT
- Antibiotics, current on synthesis nucleic acids
 - erythromycin
 - oleandomycin
 - rifampicin
 - lincomycin
- Sensitivity to antibiotics determine:
 - method membrane filters
 - method paper disks
 - two-phase fermentation method
 - sedimentation method
 - aspiration method.

Practical occupation #11

Subject: Symbiosis. Residents and pathogens. Fungi-causative agents of mycoses. Educational goal:

1. Explore stages and factors symbiosis human With microbes.
2. Study the microflora body human
3. Explore mushrooms - pathogens of mycoses and mycological method research

The student must know:

1. Stages and factors symbiosis human With microbes.
2. Microflora body person.
3. Terms formation associations residents.
4. Differences pathogens from residents.

Student should be able to:

1. Conduct sowing material with fingers hands on cup with MPA (method prints).
2. Sowing detachable from the nose and pharynx on MPA.

Plan lessons:

1. Stages and factors symbiosis human With microbes.
2. Terms formation associations residents.
3. Differences pathogens from residents.
4. What methods can study microflora human?
5. Composition resident microflora skin covers person.

Independent student work:

1. Cooking smears from yeast mushrooms, paint them simple method (methylene blue) and microscoping.
2. Cooking and microscopy native drugs from cultures moldy mushrooms.
3. View and sketch demonstration drugs:
 - a) actinomycetes, painted on Gram;
 - b native preparations from cultures of mold fungi (*mucor*, *aspergillus*, *penicillium*); in yeast mushrooms, painted methylene blue;
4. Sowing material with fingers hands on cup with MPA (method prints).
5. Sowing separated from nose and pharynx on MPA.

INFORMATIONAL MATERIAL ON THEME

Microorganisms are in various relationships with each other. A joint Existence two various organisms called *symbiosis*. Distinguish some options useful relationship: metabiosis, mutualism, commensalism, satelliteism.

Antagonistic relationship expressed in form unfavorable the impact of one type of microorganism on another, leading to damage and even death the last one. Forms antagonism: competition, predation, parasitism.

Microflora of the human body

The human body is inhabited approximately 500 types germs, constituents his normal microflora, in form communities microorganisms (*microbiocenosis*). They are in able equilibrium (*eubiose*) friend With friend and organism person. Distinguish normal microflora various biotopes: skin, mucous shells cavities mouth, top respiratory ways, gastrointestinal tract and urogenital systems. AT body allocate permanent and transient microflora. *Permanent* microflora is represented microorganisms that are constantly present in the body. *The transient* microflora capable to long existence in body. Permanent microflora can

divide on obligate and optional. obligate microflora (bifidobacteria, lactobacilli, peptostreptococcus, intestinal wand and etc.) is basis microbiocenosis, and facultative microflora (staphylococci, streptococci, Klebsiella, clostridia, some mushrooms and etc.) includes lesser part microbiocenosis. microorganisms, constituents normal microflora, concluded in highly hydrated exopolysaccharide nomycin matrix, forming biological film, resistant to various influences.

Protocol research

No.	researched material	results research	Graphic image

Mushrooms (fungi, mycetes) — heterogeneous group eukaryotic microorganisms. Fungi have a nucleus with a nuclear membrane, a cytoplasm with organelles, a cytoplasmic membrane (which contains phospholipids and sterols) and powerful cellular wall consisting of glucan, cellulose, chitin, protein, lipids, etc. Mushrooms consist of long thin threads (gif), intertwined in mycelium, or mycelium. gifs lower mushrooms — phycomycetes - do not have partitions. In higher fungi - eumycetes - hyphae are divided partitions; their mycelium multicellular. Mushrooms multiply disputes sexual and asexual ways, a also vegetative through (budding or fragmentation gif). Mushrooms, breeding sexual and asexual by, relate to perfect. Imperfect mushrooms are called mushrooms that do not have or have not yet described the sexual tract. breeding. Asexual reproduction is carried out in fungi using endogenous spores, maturing inside round structures — sporangia, and exogenous dispute — conidium, emerging on tips fruitful hyphae. Fungi can be divided into 7 classes: chytridiomycetes, hyphochytridiomycetes, oomycetes, zygomycetes, ascomycetes, basidiomycetes, deuteromycetes. overwhelming majority fungi that cause diseases in humans (mycoses) are imperfect fungi. For diagnostics mycoses may to be used microscopic (cultural), allergic, serological, biological and histological methods research. Material for research may to be pus, sputum, affected hair, nails, skin flakes, bone marrow punctates, lymph nodes, internal organs, blood, bile, feces, tissue biopsy specimens, etc. Most often used for staining smears methods Grama, Ziel-Nielsen, Romanovsky-Giemsa

TEST TASKS

- Representatives resident microflora skin human are
 - a) staphylococci;
 - b) streptococci;
 - c) lactobacilli;
 - d) yeast-like mushrooms.
- At healthy person sterile are the following bodies
 - a) kidneys;
 - b) uterus;
 - c) bronchi, lungs; d) stomach.
- At weakening organism on skin the number of
 - a) Gr - bacteria;
 - b) Gr + bacteria
- Normal microflora body human performs the following functions
 - a) protective;
 - b) transport;
 - c) immune;
 - d) respiratory.
- To useful options relationships between microorganisms relate
 - a) metabiosis;
 - b) competition;
 - c) commensalism;

D) parasitism.

Practical occupation #12 CHANGE MODULE

Practical occupation #13

Theme: Physiological mechanisms immunity. Reactions immunity (agglutinations and precipitation).

Educational goal:

1. will study physiological mechanisms immunity.
2. Explore serological methods laboratory diagnostics.

The student must know:

1. Statement of the agglutination reaction (detailed).
2. Staging reactions precipitation, practical application.
3. Receipt diagnostic serums, classification.

The student must be able to:

1. Put indicative reaction agglutination on subject glass.
2. Put extended reaction agglutination.
3. Put reaction ring precipitation.

Plan lessons

1. Antigens their nature. Types. Antigens bacteria.
2. Antibodies, classification. Structure immunoglobulins, main classes.
3. humoral and cellular immune response
4. Serological reactions, their essence and mechanism, practical application. Serodiagnostics. Seroidentification.
5. Agglutination reaction, staging methods, reaction phases, practical application.
6. Reaction precipitation, ways performances, practical application.
7. diagnostics, classification, application.
8. Diagnostic serum, receiving and kinds diagnostic sera - agglutinating (adsorbed and non-adsorbed, mono- and polyvalent), precipitating.
9. Demonstration deployed reactions agglutination, reactions hemolysis.
10. staging reactions ring precipitation.
11. Demonstration diagnostics and diagnostic sera.

Independent Job students:

1. staging and accounting indicative reactions agglutination on glass slide to identify isolated pure culture _ Gram-negative sticks.
2. Statement and accounting of an extended agglutination reaction in order to serodiagnosis abdominal typhus.
3. Setting up and taking into account the reaction of thermophilic precipitation in order to seroindication Siberian ulcers.

INFORMATIONAL MATERIAL ON THEME LESSONS

Under **immunity** (from LC. immunitas — release, deliverance from something) in biology and medicine understand the complex of body reactions aimed at preserving its structural and functional integrity when exposed to the body genetically alien substances How incoming from the outside So and formed inside organism.

Distinguish some major species immunity:

-*Hereditary immunity* (congenital, species) conditioned development in process phylogenesis genetically fixed immunity kind to given antigen or microorganism.

-*Acquired immunity* specific and not transmitted on inheritance. He formed naturally and

created artificially. Natural acquired immunity appears after an infectious disease (smallpox, measles, etc.). Artificial acquired immunity arises at vaccination.

Immunity it happens *active* and *passive*. *Active immunity* produced organism in result impact antigen on immune system (for example, at vaccination). *Passive immunity* is caused by antibodies transmitted from the immune system. mothers to kid at birth or through introductions immune serums, a same at transplantation of immune cells.

Active immunity can be *humoral* (caused by antibodies), *cellular* (conditioned immunocompetent cells) and *cellular humoral* (conditioned and antibodies, and immunocompetent cells). For example, antitoxic immunity to botulism and tetanus is humoral So How he conditioned antibodies circulating in the blood immunity to leprosy or tuberculosis is cellular, and to smallpox - cellular humoral.

sterile immunity , which persists in the absence of a microorganism, and *non-sterile*, which exist only at availability pathogen in body. Classic example non-sterile immunity is immunity at tuberculosis.

Separately allocate So called *local immunity*, which protects individual plots organism, for example, mucous shells from pathogens infectious diseases. It is formed with the participation of secretory immunoglobulin A and characterized more active phagocytosis.

Antigens — this is any genetically alien for given organism substances (usually biopolymers), which, having entered the internal environment of the body or formed in body, cause reciprocal specific immunological reaction: synthesis antibodies, the appearance of sensitized lymphocytes or the development of tolerance to this substance hypersensitivity delayed or immediate types, immunological memory.

Antigens possess specificity which tied With certain chemical group in composition molecules, called determinant or epitope. determinants antigen are those parts of it that are recognized by antibodies and immunocompetent cells.

Distinguish between *complete* and *defective (haptens) antigens*. The antigens that cause full-fledged immune answer, having 2 and more determinants, called *complete*. it organic substances microbial, vegetable and animal origin. *Haptens* can be small molecular weight chemicals or more complex chemical substances not possessing properties full-fledged antigen: some bacterial polysaccharides, polypeptide tuberculosis sticks (RRD), DNA, RNA, lipids, peptides. *Haptens* due to small molecular masses not are fixed by immunocompetent cells of the macroorganism and cannot cause a response immunological reaction. *Semi-haptens* — inorganic radicals (iodine, bromine, nitro group, nitrogen and etc.), joined to protein molecule, may change immunological specificity squirrel.

Antibody formation. In response to the introduction of an antigen, the immune system produces antibodies — proteins, capable specifically unite With antigen, caused them education and, so the way participate in immunological reactions. Relate antibodies to γ -globulins, i.e., the least mobile fraction of proteins in an electric field blood serum. In the body, γ -globulins are produced by special cells - plasma mocytes. In accordance with the International Classification of γ -globulins that carry the functions antibodies are called immunoglobulins and are denoted by the symbol Ig. Consequently, antibodies — this is immunoglobulins, produced in answer on introduction antigen and capable of specifically to interact With this same antigen.

Functions of antibodies. The primary function of antibodies is the interaction of their active centers with complementary determinants of antigens. Secondary function of antibodies includes from them capabilities:

- bind antigen With goal his neutralization and elimination from organism;
- participate in recognition "foreign" antigen;
- provide cooperation immunocompetent cells (macrophages, T- and AT- lymphocytes);
- participate in various forms of the immune response (phagocytosis, killer function, immunological tolerance, immunological memory, hypersensitivity immediate type, hypersensitivity delayed type).

Proteins of immunoglobulins are chemically classified as glycoproteins, since consist from protein and sugars; built from eighteen amino acids. Distinguish five classes immunoglobulins:

IgM, IgG, IgA, IgE, IgD. Immunoglobulins M, G, A have subclasses. For example, IgG It has four subclass (IgG1, IgG2, IgG3, IgG4).

Immunological memory is the body's ability to re-meeting with the same antigen to react more actively and more rapidly immunity those. to react on type secondary immune response.

Immunological tolerance phenomenon opposite immunological memory. In this case, in response to the repeated introduction of the antigen, the body, instead of vigorous workings immunity shows unreactivity, not answers immune reaction t. e. tolerant antigen.

I. Agglutination reaction on subject glass

Apply on a glass slide at a sufficient distance friend from three friends drops: physiological solution, typhoid agglutinating serum (No. 1) and dysenteric agglutinating serum (No. 2). Add the culture under study to the drop physiological solution and thoroughly grind in her before appearance expressed turbidity. Transfer the prepared suspension with a bacterial loop into serum No. 1 and thoroughly mix. Further bacteriological loop necessary sterilize calcination. Then take the bacterial loop material from the culture suspension in a drop physiological saline and add it to a drop of serum No. 2. Glass lightly and carefully shake to thoroughly mix. Accounting for the results of the reaction is carried out after 1-2 minutes: uniform turbidity remains in a drop of saline, then How in drop one from sera celebrated agglutination. Signs agglutination are: precipitation of grains of agglutinate and enlightenment of the liquid. If found in control drop With physiological solution spontaneous agglutination results reactions are not subject to further accounting, a herself reaction requires repeated staging.

II. deployed reaction agglutination

deployed agglutination reaction was set to determine the antibody titer in blood serum sick.

The test serum is diluted 50 times with saline, and the resulting so way breeding (1:50) counts original. Further initial breeding serum sequentially diluted twice with saline. For this (see diagram productions):

a) in all agglutination tubes, except for No. 6, 1.0 ml of physiological solution;

b) 1.0 ml of serum is added to test tubes No. 1 and No. 6 in the initial dilution of 1:50, and, so the way serum in vial no. 1 getting divorced twice more, that is in one hundred once;

c) 1.0 ml of serum from tube No. 1 is transferred to test tube No. 2 to those available in her 1.0 ml of saline, as a result of which the serum is diluted two more times, that is in 200 once, and So Further, up to before test tubes No. five, where breeding reaches 1:1600;

d) it is obvious that tubes No. 1 - No. 4 contain 1.0 ml of serum, while vial 5 contains 2.0 ml of ee - the excess 1.0 ml is removed, and thus volumes in test tubes No. 1 - No. 5 are equalized. In test tube No. 6, serum control. Further, in each test tube, with the exception of test tube No. 6, add 2 drops DIAGNOSTICUM — processed formalin suspension in physiological cell culture solution Salmonella typhi, in every milliliter of which contains 2 billion bacterial bodies. The test tube rack is shaken and placed in a thermostat at t 37°C for 2 hours. After exposure in a thermostat, the reaction rack is kept at room temperature or "in the cold" (+3° +5°C) in for 18 hours.

Components reactions	Exper ience					serum	diagnosticia n crazy
	1	2	3	four	five		
1. Phys. Solution	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2. Researched serum (1:50); ml	1.0 1:100	1.0 1:200	1.0 1:400	1.0 1:800	1.0 1:1600	1.0 1:100	1.0
3. Diagnosticum, drops	2	2	2	2	2	-	2

The results are recorded in a day in the following sequence: first turn evaluate the state of control test tubes (No. 6 and No. 7), second turn —experienced. AT test tube #6 (control serum) must to be absolutely transparent, devoid of any

sediment liquid. AT test tube No. 7 (control diagnosticum)

- Uniform haze. The results of test tubes should be evaluated starting from test tubes with the highest serum dilution (No. 5). The result of the reaction is taken into account according to fallout on bottom test tubes flakes agglutinate and simultaneous enlightenment content test tubes; at lung tapping on wall test tubes or careful agglutinate is easily separated from the bottom by shaking, floats up and, without changing its structure, returns in initial position.

III. Reaction ring precipitation

The precipitation reaction is most often used to determine the presence in the material soluble antigens. into a control precipitating tube, up to approximately half of its volume is brought in by normal serum. The same amount of precipitating serum. Next, a small amount is added to each tube. the amount of material to be tested - for example, an extract from the skin of an animal (sheep), died presumably from anthrax. The material to be tested should be through cautious layering on internal wall precipitation test tubes, held in the hand at a height of 30-35 cm from the surface of the desktop at an angle of 45 ° to horizontal.

AT experienced test tube on border serum and researched material observed education precipitate: whitish "disk" irreversibly collapsing at shaking test tubes. AT control test tube education precipitate not observed.

IV. Reaction indirect (passive) hemagglutination (RITA)

RPGA is based on the use of erythrocytes with adsorbed on their surface antigens (erythrocyte diagnosticum), the interaction of which with the corresponding antibodies in the blood serum of patients causes erythrocytes to precipitate to the bottom test tubes (wells) in form "revealed umbrella."

The studied serum of the patient is diluted 10 times and heated at 65 ° C for 20 minutes for water bath for removal non-specific hemagglutinins, then cook row her dilutions from 1:100 to 1:3200 and poured into wells of 0.5 ml. Add to each well 0.5 ml diagnosticum. The corresponding erythrocyte is added to each row of wells. diagnosticum: to shigella Sonne, Flexner, Newcastle and polyvalent salmonella.

Simultaneously put controls diagnosticums and control researched serum. The result of the reaction is taken into account after incubation in a thermostat for 2 hours at 37°C or at room temperature for 1824 hours. The reaction is considered positive when condition location erythrocytes in the form of an umbrella all surfaces hole bottom and evaluated like "+".

Scheme productions

Breeding researched serum	DIAGNOSTICS				CONTROL				
	Sonne	Flexner	New castle	Salmon. watered.	cd 1	cd 2	cd 3	cd four	Ks
1:100									
1:200									
1:400									
1:800									
1:1600									
1:3200									
Incubation at t 37 ° C; 24 hours.									
Accounting results									

TEST TASKS

1. What Components participate in reactions indirect hemagglutination?

- a) antibodies, antigens, complement;
 - b) antibodies, antigens, physiological solution;
 - c) antigens, physiological solution;
 - d) antigens, erythrocytes, antibodies, physiological solution.
2. What Components participate in precipitation reactions?
- a) corpuscular antigens, antibodies, physiological saline;
 - b) soluble antigens, antibodies, saline;
 - c) antigens, antibodies, complement;
 - d) antigens, antibodies, erythrocytes, physiological solution.
3. What components are involved in the hemagglutination inhibition reaction?
- a) antigens, antibodies, physiological solution;
 - b) antigens, antibodies, complement;
 - c) corpuscular antigens, antibodies, physiological solution;
 - d) viruses, erythrocytes, antibodies;
 - e) bacteria, erythrocytes, antibodies.
4. Additional component (Besides antigens and antibodies) participating in reactions agglutination is:
- a) complement serum nautical pigs;
 - b) isotonic solution NaCl;
 - c) erythrocytes;
 - d) hemolytic system.
5. Visual result reactions agglutination:
- a) hemolysis erythrocytes ram;
 - b) delay hemolysis erythrocytes ram;
 - c) enlightenment cloudy environments reactions and education coarse (grainy) draft;
 - d) turbidity transparent environments reactions and education finely dispersed suspension (flocculate) or rings precipitation.

Practical occupation #14

Theme: Serological reactions. Reactions immunity With participation complement.

Reactions immunity With labeled components.

Educational goal:

1. Explore complement dependent serological reactions,
2. Explore reactions immunity With labeled components.

The student must know:

1. staging reactions immune lysis. 2. Staging reactions binding complement (RSK).
3. staging reactions immunity With labeled components.

The student must be able to:

1. Put reaction immune lysis.
2. Put reaction binding complement (RSK).
3. Put reaction immunity With labeled components.

Plan lessons:

1. Reactions immune lysis, Components.
2. Reaction hemolysis.
3. Reaction binding complement (RSK). Staging and accounting reactions binding complement.
4. Reaction immunofluorescence, straight and indirect.
5. ELISA analysis, Components, application.
6. radioimmune analysis, Components, application.

Independent work of students: 1.

Setting and accounting for the complement fixation reaction with the goal serodiagnosis syphilis/

INFORMATIONAL MATERIAL ON THEME LESSONS:

principled scheme productions reactions binding complement

Components reactions	tube number		
	1(exp)	2 (counter.)	3 (counter.)
1. Researched serum (1:5)	0.5	0.5	-
2. Antigen in working dose	0.5	-	0.5
3 Complement in working dose	0.5	0.5	0.5
4. Physiological solution	-	0.5	0.5
Incubation at t 37 °C - 40 minutes.			
5. Hemolytic system (erythrocytes sheep + hemolytic serum)	1.0	1.0	1.0

Incubation at t 37 °C — 40 minutes.

Accounting results Hemolysis + Hemolysis +

Conclusion:

At availability antibodies in researched serum (positive reaction) in experienced test tube hemolysis absent. At negative reactions (No antibodies) in all three test tubes observed hemolysis.

The complement fixation reaction takes place in two phases: 1st phase - interaction researched serum With antigen and complement. 2nd phase — indicator — determination of the presence of free complement in the mixture by adding hemolytic systems, consisting from erythrocytes ram and hemolytic serum, containing antibodies to erythrocytes ram. If in first phase reactions going on education antigen-antibody complex, complement is bound by this complex in the second phase hemolysis erythrocytes absent (reaction positive). If antigen and antibody not correspond friend friend, complement in the first the reaction phase remains free and during second phase reactions joins to complex erythrocyte-hemolytic serum, causing hemolysis (reaction negative).

TEST TASKS

Specify correct answers:

1. Varieties of the immune lysis reaction are:

- a) hemagglutination;
- b) hemolysis;
- c) ring precipitation;
- d) bacteriolysis;
- e) cytolysis.

2. What kind more Components (Besides antigens and antibodies) participate in reactions binding complement?

- a) bacteria;

- b) complement;
- c) erythrocytes ram;
- d) hemolytic serum;
- e) physiological solution.

3. When setting up an enzyme immunoassay, antibodies or antigens are labeled:

- a) peroxidase;
- b) fluorochrome;
- c) a radioisotope;
- d) complement.

4. Most modern serological reactions are:

- a) reaction agglutination;
- b) complement fixation reaction;
- c) enzyme immunoassay analysis;
- d) polymerase chain reaction;
- e) reaction immunofluorescence.

5. To identify the presence of a site characteristic of a particular microorganism nucleic acid in researched material and repeatedly propagate his allows:

- a) enzyme immunoassay analysis;
- b) polymerase chain reaction;
- c) radioimmune analysis;
- d) reaction immunofluorescence.

Practical occupation #15

Topic: Immune status

Educational goal:

1. Explore tests first and second level, them clinical interpretation.

The student must know:

- 1 Age peculiarities immune status.
- 2. Methods research lymphocytes, assessment functional states phagocytes,

The student must be able to:

- 1. Decide and take into account the functional condition phagocytes,
- 2. Define activity complement blood

Plan lessons:

- 1. Immune status and principles his estimates.
- 2. Age peculiarities immune status.
- 3. Methods research lymphocytes, grade functional state phagocytes,
- 4. Definition complement
- 6. Tests first and second level, them clinical interpretation.

Independent Job students:

- 1. staging and functional state of phagocytes,
- 2. Complement determination INFORMATION MATERIAL Maturation immune reactivity fetus

thymus is being laid on second month intrauterine life in areas third- fourth gill pockets and on sixth week It has expressed epithelial character. On 7-8 week he colonized by lymphocyte-like cells. To end third month, the formation of the organ ends. Later in the thymus, there are only quantitative changes. Lymphatic nodes and other secondary organs of the immune system systems are laid on 4th month, them final formation ends in postnatal period. Lymphoid follicles located in the ileum and appendix, in Peyer's patches contain "cells predecessors" plasmatic cells. They are ripen before plasma cells, synthesizing IgA to 14-16 week intrauterine development fetus.

Stem cells appear at 3-8 weeks of embryogenesis and are found in the liver, blood islands of the yolk sac. Later, their main place of education becomes Bone marrow. Lymphocytes are first detected at week 9 in the thymus, at 12-15 spleen. In the blood, lymphocyte-like cells are determined from 8-10 weeks. Lymphoid cells, endowed function T-lymphocytes, come to light on 10-11 week. B cells determined in liver With 10- 12, in spleen — With 12 weeks. Synthesis and secretion IgM is registered in cells on the 11th, IgG - on the 22nd week. The content of IgM is 1/10 of maternal, a IgG — more smaller. Education components systems complement starts at fetus on 8th week pregnancy. At this Components C2 and C4 synthesized macrophages, C5 and C4 - in liver, lungs peritoneal cells NW and C1 — in thin and thick gut. On 18th week development Everybody specified Components determined in serum blood fetus. Cellular and humoral factors non-specific anti-infective immunity appear in early ontogeny

During the period of embryonic development, the "work" of the immune system has its own characteristics. AT in particular among T-dependent immunological reactions, the ability to to rejection transplant (13 a week), HRT implemented much later.

Despite on Availability in body fetus significant quantities B cells With immunoglobulin receptors plasmatic cells, directly synthesizing AT, very few. Row very powerful factors suppresses function humoral part of the immune system. It is a choriotropic gonadotropin, a-fetoprotein, a-2-globulin. Sharp limited in this period influence on B-cell- ki T-lymphocytes and macrophages.

Premature activation immune systems observed at intrauterine infection. Practically always this is accompanied by any immunopathological disorders. So the way for embryonic period a typical step in immunogenesis is the tolerance of one's own immune system and passive antibody immunity per check maternal IgG, concentration which progressively is growing in process pregnancy. Ability fetus form Components systems complement defective. AT III trimester her level although and increases but is not more 30-50% indicators adults. System local immunity in early and late ontogeny not developed.

immune status at children after birth

A healthy full-term baby born to a healthy mother with a physiological course pregnancy, It has definite immune status and corresponding level factors nonspecific anti-infectious resistance. Peculiar character passive immunity of the newborn has positive and negative sides. So, not receiving from mothers IgM, fetus not saturates related With this class group isohemagglutinins, what reduces risk development conflict at mismatch group erythrocyte Ag. FROM another sides induced low protection against gram-negative bacteria, since this fraction predominantly are AT against the specified pathogens. At the time of birth, the child has physiological leukocytosis, reaching before 12-15 billion cell/l. From cells more 35% constitute lymphocytes. From general numbers lymphocytes about half of constitute T-cells. In relative terms, their content moderately reduced, and in absolute terms, Considering tall leukocytosis, not changed. About 60% all T-lymphocytes constitute cells With helper functions, 15% — T-suppressors.

Content antibody-dependent killers same strongly reduced from level adults. Functions lymphocytes newborns changed. So, intensity reactions blast transformation induced by PHA T-mitogen is "normal" or slightly reduced, how at more senior children. reduced them ability produce lymphocytes,

induce skin reactions. At the same time, the cells show a higher level metabolism, if judge on intensity nucleic synthesis acids.

The number of B cells in newborns is usually increased in relative and absolute values. As rule on these cells are found IgM and IgE receptors. AT cord blood of newborns, IgM and IgG are detected, IgA and IgE are detected rarely. The synthesis of IgM increases sharply, reaching a maximum at 2-3 weeks of a child's life, then by the age of one month it decreases, then slowly increases, reaching by 6-12 adult level. An excessive increase in the concentration of IgM in newborns is evidence of intrauterine infection. Most often it is syphilis, rubella. A three-fold increase in IgM levels is evidence of sepsis in child.

The concentration of IgG is very low at birth, increases by 7-8 years. In children fed artificially, this dynamics implemented much faster. IgA in serum blood newborns, How rule missing in flow first months life. In the future, the content of immunoglobulin slowly increases, reaching by the end the first year 28% of the level of this protein in adults. Normalization of the parameter is achieved by 8-15 years. IgD at newborns usually not is determined. Appears this protein approximately on 6th week, reaching level adults to 5—10—15 years. IgE same not is found at newborns, gradually growing, he approaching to values adults by the age of 11-12. Acceleration of reagin accumulation is a risk of development in children bronchial asthma and others allergic and especially atopic diseases.

It is known what content immunoglobulins determined sum AT various specificity. Earlier than others in children, the appearance of immune globulins has an effect microflora organism child. Main representative intestinal microflora in this

period are bifidumbacteria. therefore any unfavorable factors (artificial feeding, application antibiotics) inevitably entail per yourself violation specific composition microflora and changes spectrum emerging C. Antibody formation in newborns, as a rule, proceeds only according to the primary type, requiring for the implementation of a large amount of Ag. Significantly slow switchingsynthesis with IgM on IgG, in flow 5-20 days at adults and 20-40 — at children.

At the time of birth, phagocytes and blood serum of newborns have a certain bactericidal activity vs row microbial strains. Chemotaxis and functional activity macrophages reduced. Partially this is compensated increase content granulocytes, So same endowed phagocytic function. However, digestion ability these cells lowered per check enzyme immaturity.

A child is born with reduced complement levels compared to adults. properdin, which grow quite quickly. The initial activity of lysozyme, on the contrary, significant.

Content lysozyme in body not always, depends from age, time of the year, vitamin balance and others More Total lysozyme in saliva children (before 200 mcg/ml), what in many times higher than its concentration in blood serum. The highest content lysozyme in the saliva of children of the first year of life, at the age of 1-6 years it decreases by almost 3 times, to 7-15 years increases but not reaches original level. Important factor local immunity is IgA, which is in two forms - serum and secretory. This y-globulin plays a major role in the body's resistance against respiratory, viral, bacterial, parasitic infection, etc. Secretory IgA starts show up in secrets first and early second weeks continues progressively increase in the following months and years, in coprofilters it is found with third week of life. The amount of secretin is constantly replenished due to the secretory IgA of milk and, especially, colostrum, where its amount is 20 times or more higher than the level in adult serum. Usually after 3-5 days of lactation, the concentration of IgA decreases sharply, but, given the increasing consumption of milk by the child, its amount Plasma cells located in the mucous membranes form IgA, IgM, IgG, IgD, IgE. Wall intestines synthesizes before 3 G immunoglobulins in day. IgG provide protection in mostly vs toxins rest vs bacteria and viruses. Formation full-fledged local immunity, according to various sources, ends by one to twelve years life.

Ratio plasmatic cells gastrointestinal track, producing immune globulins, changes in some diseases. Yes, in young children (from birth and up to three years old) With chronic gastroduodenitis there is an IgA deficiency and

increase products IgM. At patients With cholecystitis celebrated decrease IgA concentrations and an increase in IgM or IgG. With peptic ulcer of the duodenum 12 going on the fall level IgA in duodenal content. deficit local IgA facilitates binding immune globulins others classes With Ag.

Local immunity conditioned not only humoral, but and cellular factors. Shown what in the first 24 hours after birth baby going sharp increase in the number of alveolar macrophages. Their number continues to increase monthly

age, after what stabilizes. Microbicidal properties macrophages and others phagocytic cells, How rule behind at children first weeks and even months life.

State immune systems child in first years life characterized high dynamism. So, after birth declining

number leukocytes in circulation, rises percentage content lymphocytes, decreased number granulocytes.

The crossover between the curves reflecting the dynamics of these cells occurs for the first time at 5 day of life, after which a similar cross (decrease in the proportion of lymphocytes and increase in neutrophils) celebrated only in age 4-5 years old. Highly slowly

risers relative content T cells level B-lymphocytes steadily declining before norms.

Thus, for the embryonic period, tolerance and passive immunity per check maternal IgG, concentration which is growing in process pregnancy. In the newborn, maternal passive immunity also dominates, although already celebrated Start synthesis own AT, endowed small 12 months immune reactivity matures. AT age 1-3 years old distinctly working T cell immunity. AT this same period active are functioning and B-lymphocytes.

From the foregoing it follows that the body of a newborn up to one year of age is badly protected from infectious agents. Active main way humoral link immunity. T-dependent reactions and phagocytosis developed not enough and enter in complete force later. Sometimes only to period of sexual maturation. Considering Everybody these intelligence the appointment of children with immunotropic drugs should be done with extreme caution so that do not distort the natural features of the reaction, mistaking for immune disorders physiological changes immune reactions.

In many diseases in children, the liver and spleen. These bodies in intrauterine period carry out hemo- and lymphopoiesis. therefore in answer on damage or infection fetus answers activation reticuloendothelial system. After birth, its significance decreases, being replaced by more committed mechanisms. However, at parts So called "slowly starting children" With delay maturation immune systems possible reaction on pathogenic situation specified organs.

At present, there are several critical periods in the life of a child, which characterized greatest vulnerability organism (D.V. Stephanie, Yu.E. Veltishchev, 1996).

In intrauterine period critical should think age 8-12 week, when differentiation of organs and cells of the immune system occurs. First critical the period after birth is the neonatal period, when the body is exposed to action huge numbers Ag. immune system in this is time exposed strong suppressive influences, passive humoral immunity is due to maternal C. There is a functional imbalance of T-lymphocytes, the suppressor function is realized Not only CD8⁺ cells, but and immature thymocytes and other cells.

Second critical age (3-6 months) characterized weakening passive humoral immunity due to the catabolism of maternal C. At the same time, the suppressor the direction of immune peak is consistent with the presence of pronounced lymphocytosis. Such type immune response comes at vaccination vs tetanus, diphtheria, whooping cough, poliomyelitis, measles, and only after the 2-3rd revaccination does secondary immune answer With education IgG AT and persistent immune memory.

The third critical period is the 1st year of life. At this time, the primary character is preserved immune response to many antigens, however, it is already possible to switch to the formation of IgG- C. However synthesis subclasses IgG2 and IgG4 is late. suppressor orientation immune mechanisms begins to be replaced by helper. Local immunity is not developed, children sensitive to respiratory viral infections. Fourth the critical period is the 4th-6th years of life. At this age, the average concentration of IgG and IgM in the blood corresponds to that in adults, the concentration of IgA in plasma does not yet reach final values, the content of IgE in the blood reaches its maximum values. The period is characterized by a high frequency of atopic, parasitic, immunocomplex diseases.

The fifth critical period is adolescence (in girls from 12-13, in boys from 14-15 years old). Pubertal jump growth combined With decrease masses lymphoid organs. Raise secretions genital hormones (before Total androgens) leads to suppression of the cellular link of immunity and stimulation of its humoral mechanisms. AT In general, children have the following features of the immune status links. T - link immunity. The number of peripheral blood lymphocytes at birth on the first day life is 24-30%, and the absolute number - $3-9 \cdot 10^9$ /l. Then their relative the number is growing and to 4th-

5th days reaches 40-50%, absolute — 2.5— 10 billion/l

Lymphocytes newborns different high metabolic activity, in them increased DNA synthesis. and RNA. BTL during cultivation with PHA is well expressed as in full-term, So and at premature newborns. noted tall level spontaneous transformation, on average, about 6-10%, while in adults this figure is about 0.2%. AT — link immunity. System humoral immunity in difference from cellular starts actively function only after birth under influence antigenic irritation. At birth child content IgG in his blood usually more than the mother, since the transplacental passage of this immunoglobulin is active process. IgM in serum usually missing or determined in minimal quantities. IgA usually missing or are in trace concentrations. By the end of the first week, the content of IgA and IgM increases slightly, IgG - by the 2nd-3rd week it noticeably decreases and reaches the minimum concentrations at the age of 1-4months

phagocytic unit. The number of neutrophils in the blood at birth is relatively high: 50- 70% and 4.5-20 billion/l. From the 4th day, it begins to decrease to 30-40% - 2.5-6 billion / l. Monocytes during the entire period of the newborn TM make up 4-9% - 0.6-2 billion / l. Absorption ability neutrophils newborns not lowered but digestion activity is reduced, which leads to incomplete phagocytosis. Number HCT-positive neutrophils in spontaneous reactions at children first 2 weeks life is 14-20%, while in older children it is 2-10%. The rise in the number of these cells in the induced test is low, i.e. phagocytic reserve of cells in children aged two weeks small. Monocytes newborns characterized low bactericidal activity and insufficient migratory ability.

SITUATIONAL TASKS

CLASSES IMMUNOGLOBULIN

IgA , Ig M, IgF, IgE , IgD
IgA , IgM , IgG , IgE , IgD (+)
IgA , Ig M, IgG , Ig E, IgF
IgM, IgG, IgE, IgF, IgD
IgA , IgG , Ig E, IgF, IgD

TALL LEVEL GENERAL IgE CHARACTERIZES

helminthiases, allergy
allergies, autoimmune diseases
helminthiases, immunodeficiencies
immunodeficiencies, allergies
helminthiases, viral infections

CLINICAL MANIFESTATIONS OF C -4 COMPONENT DEFICIENCYCOMPLEMENT

rheumatoid arthritis
tuberculosis periodic
illnessalveolitis
SLE

CASCADE SYSTEM SERUM BLOOD, CAPABLE CALL LYSISCELLS, THIS IS

complement system
cytokine network
interferons
kalecrein-kinin system
immunoglobulins

At SICK ALLERGY To YODUS, TO HIM CONTRAINDICATED

butadione
brufen
enteroseptol

Practical occupation #16

Topic:

Immunodeficiencies

Educational goal:

1. Explore pathogenesis secondary immunodeficiencies
2. Explore genetics immunodeficiencies, peculiarities inheritance.
3. Explore congenital immunodeficiencies

The student must know:

1. genetics immunodeficiencies, peculiarities inheritance.
2. Secondary immunological deficiency (SID) - classification, etiology, diagnostics

The student must be able to:

1. Estimate and interpret indicators immune status at secondary immunological insufficiency

Plan lessons

1. Genetics immunodeficiencies, peculiarities inheritance.
2. Congenital immunodeficiencies (classification, diagnostics)
3. Congenital immunodeficiencies at children.
4. Secondary immunological deficiency (SID) - classification, etiology, diagnostics

Independent Job students:

1. Estimate and interpret indicators immune status at secondary immunological insufficiency on ready immunograms

INFORMATIONAL MATERIAL

Immunodeficiencies (IDS) — violations immunological reactivity due to fallout one or several components immune apparatus or closely interacting With him non-specific factors.

United classification not exist. By origin immunodeficiencies divide on primary and secondary. Contents [put away]

- 1 Primary immunodeficiencies
 - 1.1 Definition and classification
 - 1.2 Clinical painting IDS
 - 1.3 Treatment primary IDS
- 2 Secondary immunodeficiencies
 - 2.1 Causes
 - 2.2 Treatment secondary IDS

Definition and classification

Primary immunodeficiencies - this is congenital (genetic) or embryopathies) defects immune systems. AT depending on level violations and localization defect they there are:

humoral or antibody — With predominant defeat systems AT- lymphocytes)

X-linked agammaglobulinemia (Bruton's disease)Hyper-

IgM syndrome

X-linked autosomal

recessive

deletion of immunoglobulin heavy chain genes

deficit k-chains

selective IgG subclass deficiency with or without IgA deficiency

deficiency of antibodies with normal levels of immunoglobulins

general variable immune failure

IgA

deficiency

cellular
 Dee syndrome Georgie primary
 deficiency of CD4 cells deficit
 CD7 T cells
 IL-2 deficiency
 multiple cytokine deficiency defect
 transmission signal combined:
 syndrome Wiskott-Aldrich
 ataxia-telangiectasia (syndrome Louis Bar)
 severe combined immunodeficiency X-linked With floor
 autosomal recessive deficit
 adenosine deaminase
 deficit purine nucleoside phosphorylase
 deficit molecules II class WPC (syndrome bald lymphocytes) reticular dysgenesis
 CD3 γ or CD3 ϵ deficiency
 deficit CD8 lymphocytes
 insufficiency of the complement system
 defects phagocytosis
 hereditary neutropenia
 infantile lethal agranulocytosis (Kostman's disease) cyclic
 neutropenia
 familial benign neutropenia defects in
 phagocytic function chronic granulomatous
 disease
 X-linked autosomal
 recessive
 type I lymphocyte adhesion deficiency
 deficit adhesion leukocytes 2 type
 neutrophil glucose-6-dehydrogenase deficiency
 deficit myeloperoxidase
 deficiency of secondary granules
 Shwachman's syndrome Clinical
 picture of IDS Clinic has a
 number general heck:

1. Recurrent and chronic infections top respiratory ways, paranasal sinuses, skin, mucous membranes, gastrointestinal tract, often called opportunistic bacteria protozoa, mushrooms, having trend to generalization, septicemia and torpid to ordinary therapy.
2. Hematological deficits: leukocytopenia, thrombocytopenia, anemia (hemolytic and megaloblastic).
3. Autoimmune disorders: SLE-like syndrome, arthritis, scleroderma, chronic active hepatitis, thyroiditis.
4. Often, IDS is combined with type 1 allergic reactions in the form of eczema, edema Quincke, allergic reactions on introduction medicinal drugs, immunoglobulin, blood.

5. Tumors and lymphoproliferative diseases in IDS occur 1000 times more often, how without IDS. [1]
6. At sick With IDS often are celebrated disorders digestion, diarrheal syndrome and syndrome malabsorption.
7. Sick With IDS different unusual reactions on vaccination, a application at them alive vaccines dangerous development sepsis.
8. Primary IDS often fit together With vices development, before Total With hypoplasia cellular elements cartilage and hair. Cardiovascular vices described, main the way at syndrome Dee George.

[edit]

Treatment primary IDS

Etiotropic therapy is in corrections genetic defect methods genetic engineering. But this approach is experimental. Main efforts at established primary IDS directed on:

prevention infections

substitution correction defective link immune systems in form transplants bone marrow, substitution immunoglobulins, transfusions neutrophils.

enzyme replacement therapy cytokine

therapy vitamin therapy

treatment of associated infections

Secondary immunodeficiencies

Factors capable call secondary immunodeficiency, very varied. Secondary immunodeficiency can be caused by both environmental factors and internal factors organism. AT in general, Everybody unfavorable factors environmental environment, capable break exchange substances organism, may become cause development secondary immunodeficiency. To most widespread environmental factors that cause immunodeficiency include pollution environmental environment, ionizing and microwave radiation, sharp and chronic poisoning, long-term use of certain drugs, chronic stress and overwork. General trait described higher factors is complex negative impact on all body systems, including immune system. Except Togo, such factors How ionizing radiation render electoral inhibitory action on immunity related With oppression systems hematopoiesis. People, living or working in conditions polluted environmental environment, more often get sick various infectious diseases and more often suffer oncological diseases. It is obvious that such an increase in the incidence in this category of people is associated with decline activity immune systems.

Causes

Secondary immunodeficiencies are a common complication of many diseases and states.

Main causes secondary IDS:

defect nutrition and general exhaustion organism same leads to decrease immunity. On background general exhaustion organism violated Job all internal organs. immune system especially sensitive to lack vitamins, minerals and nutritional substances So How implementation immune protection is an energy-intensive process. Often a decrease in immunity is observed during seasonal vitamin insufficiency (winter spring)

chronic bacterial and viral infections, as well as parasitic infestations. (tuberculosis, staphylococcosis, pneumococcosis, herpes, chronic viral hepatitis, rubella, HIV malaria, toxoplasmosis, leishmaniasis, ascariasis and etc.). At various chronic diseases infectious character immune system undergoes serious changes: violated immunoreactivity, develops increased sensitization on relation to various antigens microbes. Except Togo, on background chronic infectious process observed intoxication organism and oppression functions hematopoiesis. Immunodeficiency in time infections HIV mediated by selective damage to cells of the immune system by a virus helminthiasis

loss of immune defense factors is observed during severe blood loss, with burns or kidney disease (proteinuria, chronic renal failure). The common feature of these pathologies is significant loss plasma blood or dissolved in her proteins, part of which is immunoglobulins

and others components immune systems (proteins systems complement C-jet protein). In time bleeding is lost not only plasma, but and cells blood, so on background strong bleeding decline immunity It has combined character (cellular humoral)

diarrhea syndrome

stress syndrome

heavy injury and operations same flow can decline functions immune systems. In general, any serious disease of the body leads to a secondary immunodeficiency. Partly this is related With violation exchange substances and intoxication organism, a partly With topics what in time injuries or operations stand out large quantities hormones adrenal, which oppress function immune systems

endocrinopathy (DM, hypothyroidism, hyperthyroidism) lead to a decrease in immunity for check violations exchange substances organism. Most expressed decline immune reactivity of the organism is observed in diabetes mellitus and hypothyroidism. At these diseases declining production energy in fabrics, what leads to violation processes division and differentiation cells, in volume including and cells immune system. Against the background of diabetes mellitus, the frequency of various infectious diseases are on the rise. This is due not only to the inhibition of the function immune systems, but and With topics what elevated content glucose in blood sick diabetes stimulates bacterial growth

sharp and chronic poisoning various xenobiotics (chemical toxic substances, medicines, narcotic drugs). Especially expressed decline immune protection in time reception cytostatics, glucocorticoid hormones antimetabolites, antibiotics

low weight body at birth

decreased immune defenses in the elderly, pregnant women and children related with age and physiological features organism these categories people

malignant neoplasms - disrupt the activity of all body systems. The most pronounced decrease in immunity is observed in the case of malignant blood diseases (leukemia) and when red bone marrow is replaced by metastases tumors. Against the background of leukemia, the number of immune cells in the blood sometimes increases tens, hundreds and thousands of times, however, these cells are non-functional and therefore cannot ensure normal immune protection organism

Autoimmune diseases occur due to dysfunction of the immune system. On background diseases this type and at them treatment immune system working not enough and, sometimes not right, what leads to damage own fabrics and inability overcome infection
Treatment secondary IDS

The mechanisms of immune suppression in secondary IDS are different, and, as a rule, there is a combination of several mechanisms, disorders of the immune system are expressed to a lesser extent than in the primary. As a rule, secondary immunodeficiencies are transitory. In this regard, the treatment of secondary immunodeficiencies much simpler and more efficient on comparison With treatment primary violations immune system functions. Treatment for secondary immunodeficiency is usually started With definitions and eliminate causes his occurrence. For example, treatment immunodeficiency on background chronic infections start With sanitation foci chronic inflammation. Immunodeficiency on background vitamin and mineral deficiencies begin to be treated with the help of complexes of vitamins and minerals. Recovery capabilities immune systems are great so elimination causes immunodeficiency, How rule leads to restoration immune systems. For acceleration recovery and stimulation immunity carry out well treatment with immunostimulating drugs. At present, it is known number of immunostimulating drugs, With various mechanisms actions.

Recommended literature:

1. Shabalov N. P. Baby disease, Peter, Medical Literature, 2000, p.989-1027
2. Long V. T. Basics immunopathology, Phoenix, Rostov-on-Don, 2007 pp.119-158
3. stephanie D. C., Veltishchev YU. E. Immunology and immunopathology, Moscow,

Medicine, 1996 p.88-170

4. Khaitov R.M., Secondary immunodeficiencies: clinic, diagnostics, treatment, 1999

6. Modern Problems allergology, immunology and immunopharmacology, M.,2002

7. Zabrodsky P. F., Mandych V. G. Immunotoxicology of xenobiotics: Monograph. Saratov, SWIBHB, 2007. 420 With. 2007

EXAMPLES SITUATIONAL CHALLENGES:

A task 1. At sick K. 15 years old in immunogram identified the following changes.

INDEX	AT RATE	At SUBJECT
CD3+lymphocytes in%	60-80	73
CD4+ lymphocytes in%	33-50	40
CD8+lymphocytes in%	16-39	29
CD16+lymphocytes in%	3-10	7
CD20+lymphocytes in%	6-23	21
Index CD4+/CD8+	1.5-2.0	1.5
phagocytic activity %	50-90	68
phagocytic number	2-9	five
phagocytic reserve %		65
IgG, g/l	0.9-4.5	6.0
IgA, g/l	8-20	2
IgM, g/l	0.6-2.5	1.8

Questions:

1. Which link immunity violated according to the results submitted immunograms?
2. Which immunological diagnosis You put sick by changes in immunogram?
3. What kind immunomodulators can appoint sick for corrections identified changes?
4. When necessary spend repeated immunological examination after immunocorrection?
5. What kind most often encountered complaints presents sick With diagnosis immunological insufficiency?

Answers:

1. humoral link immunity.
2. Hypoimmunoglobulinemia (decrease content IgA).
3. Ribomunil, Bronchomunal, IRS-19, Likopid.
4. Not earlier how through 2 weeks after graduation therapy.
5. Frequent colds diseases, prolonged flow infectious diseases, Availability diseases, caused conditionally pathogenic flora, frequent exacerbations any chronic diseases.

Task 2. A 40-year-old patient complained of episodes of sneezing (from 10 to 30 contract), on plentiful selection watery secret, leading to hyperemia - irritation of the skin of the wings of the nose and upper lip, impaired nasal breathing, itching of the nose, palate, eye, lacrimation. Data symptoms appear in summer time and most expressed With morning. Same sick notes light fatigue, absence appetite irritability.

Questions:

1. Your conjectural diagnosis?
2. Which volume allergic surveys You appoint patient?
3. What kind groups drugs shown in given clinical case?
4. AT what case You would appoint local hormonal therapy as spray?
5. Perhaps whether conducting specific immunotherapy at given sick?

Answers:

1. Allergic rhinitis.

2. General analysis blood, immunological examination, definition IgE-general, IgE-specific conducting skin samples
3. Antihistamines, mast cell membrane stabilizers , application hormonal nasal sprays, conducting SIT.
4. AT case expressed exacerbations allergic rhinitis.
5. Yes.

A task 3. Sick M, 13 years old, endured operation on about gangrenous perforative appendicitis, diffuse peritonitis. Flow postoperative period complicated lower lobe left-sided pneumonia. AT immunogram celebrated leukocytosis, lymphopenia, decline indicators CD3+cells, CD4+cells, CD8+cells, decline IRI.

Questions:

1. What is immunological conclusion?
2. Which immunocorrection in combination With therapy antibiotics shown in given case?

Answers:

1. Secondary immunological deficiency in the T-cell link. 2. Purpose T-immunostimulants, option choice is "Imunofan".

A task four. sick P., 29 years old enrolled on "03" With directional diagnosis spicys serum-like syndrome in allergic department GKB. At admission disturbed arthralgia, dyspnea, fever, cutaneous itch, congestion nose, cough co meager sputum purulent detachable from left ear.

From the anamnesis it is known that a month ago she was treated for acute purulent otitis and sore throats antibiotic augmentin in flow 7 days without effect, in flow months kept subfebrile condition, sweating, chilling, observed in polyclinic, where underwent a course of physio- and laser therapy. During the last 5 days before admission to department condition middle gravity. on skin around joints hemorrhagic rash, lymphadenitis, herpes labialis. Same at sick ulcerative necrotic stomatitis, left-sided spicy middle otitis, otomycosis, fungal defeat mucous nose and pharynx, vasculitis, arthralgia, fever, severe weakness. In blood tests, leukocytosis, hyperglobulinemia, promotion level transaminases and Sahara blood, high ESR and FROM- reactive protein, proteinuria.

Questions:

1. Your conjectural diagnosis?
2. Will whether changes in immunogram at given pathology, and which?

Answers:

1. Granulomatosis Wegener.
2. Changes in laboratory and immunological parameters in Wegener's granulomatosis testify about systemic inflammatory process and organ damage targets. specific for given pathology are ANCA - antineutrophilic cytoplasmic antibodies.

Practical occupation #17

Change module: "Infectious immunology. Reactions immunity. Immune status. Immunodeficiencies".

№ ЛД-21

**FEDERAL STATE BUDGETARY EDUCATIONAL INSTITUTION HIGHER EDUCATION
"NORTH OSSETIAN STATE MEDICAL ACADEMY»
MINISTRY OF HEALTH RUSSIAN FEDERATION**

DEPARTMENT MICROBIOLOGY

**COLLECTION METHODOLOGICAL DEVELOPMENT
ON MICROBIOLOGY, VIROLOGY AND IMMUNOLOGYFOR
STUDENTS OF MEDICAL FACULTY**

AUTUMN SEMESTER

Vladikavkaz

Author: assistant professor, PhD Chertkoeva M.G.

The main purpose of the developments is methodological assistance to students for each practical training in the fall semester. The instructions are drawn up in accordance with Federal public educational standard Supreme and professional education.

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PRACTICAL OCCUPATION No. 1.

TOPIC: STAPHYLOCOCCI. MICROBIOLOGICAL DIAGNOSIS *Staphylococcal DISEASES*.

TRAINING GOAL:

1. Explore biological properties staphylococci.
2. Explore methods microbiological diagnostics staphylococcal diseases.

Student should know :

1. Morphology, cultural, tinctorial properties staphylococci and streptococci. Enzymatic activity.
2. Factors pathogenicity and toxins. Their role in pathogenesis staphylococcal infections.
3. Main diseases called staphylococci, Pathogenesis, peculiarities immunity at staphylococcal infections. Sources and way transmission infections.
4. Principles microbiological diagnostics, basic method research, scheme identification dedicated clean culture, phage typing.
5. Specific prevention and therapy staphylococcal infections.

Student should be able to:

1. Carrying out bacteriological research (according to the scheme). Accounting and interpretation results.
2. Cooking smear and coloring on Gram.
3. Luminous microscopy drugs from pure cultures staphylococcus, streptococci.

CONTROL QUESTIONS ON THEME LESSONS:

1. Morphology, cultural and biochemical properties staphylococcus.
2. Factors virulence staphylococcus.
3. Antigens staphylococcus.
4. diseases, called staphylococcus.
5. Methods diagnostics and researched material at staphylococcal diseases.
6. Preparations for specific prevention and treatment staphylococcal diseases.

INDEPENDENT JOB STUDENTS:

1. Explore morphology staphylococcus in smear from clean culture, describe sketch.
2. To give macroscopic characteristic colonies on milk-salt agar (bacteriological method diagnostics, 1st stage research).
3. To identify culture staphylococcus on morphological, cultural, biochemical properties, define factors virulence (2nd stage bacteriological method):
 - a) recording the results of inoculation of staphylococcus culture on blood agar with a goal definitions hemolysin.
 - b) accounting results sowing in citrate plasma for definitions plasminogen activator.
 - c) accounting seeding results on yolk-salt agar for the purpose definitions lecithinases.
 - d) accounting seeding results on Wednesday With mannitol.
4. Describe drugs for specific therapy and prophylaxis staphylococcal diseases (staphylococcal toxoid, antistaphylococcal plasma, antistaphylococcal immunoglobulin, staphylococcal bacteriophage).
5. Decor protocol research.

METHODOLOGICAL RECOMMENDATIONS

The main method for diagnosing staphylococcal diseases - bacteriological. For allocation clean culture researched material sow on yolk-salt, blood or milk-salt agar. Grown isolated colonies

are subcultured on oblique agar for receiving clean culture.

Identification clean culture carry out on morphological, cultural, biochemical properties, then define factors virulence.

I. DEFINITION HEMOLYTIC ACTIVITIES BACTERIA.

A culture of staphylococcus was made on a blood agar plate. The cups are left in thermostat on 24 hours at temperature 37 degrees.

When evaluating the results, attention is paid to areas of hemolysis, i.e. enlightenment of the environment around grown colonies. Hemolytic properties bacteria connected With presence hemolysin (exotoxin).

II. DEFINITION LECITHINASES

Staphylococcus aureus was inoculated on a plate with yolk-salt agar. The cups are left in thermostat on 24 hours.

When evaluating the results, the presence of turbidity haloes around the colonies is taken into account, which testifies about education staphylococcus aureus lecithinase enzyme.

III. FOR DETECTIONS ENZYME PLASMACOAGULASES

Produce sowing culture staphylococcus in citrate plasma. test tubes put in thermostat. The results are taken into account after 24 hours. In the presence of the enzyme plasmacoagulase going on coagulation plasma With education clot fibrin. Availability enzyme plasmacoagulase is the main identification feature of the species S.aureus, which often is pathogen nosocomial infections.

IV. DEFINITION FERMENTATION MANNITOL AT ANAEROBIC CONDITIONS

To determine this feature, confirming the belonging of a pure culture staphylococcus to the most aggressive species S.aureus, sowing was done on a medium with mannitol. At cleavage of mannitol, acidic products are formed, which change the color of the indicator in environment (indicator Andrade - gives red coloring environment, a indicator VR - blue).

No P/P	researched material	results research	Graphic image

Informational material to topic

Of the 14 species of staphylococci that live on the skin and mucous membranes of humans, prevail and often cause diseases: S.aureus, S.epidermidis, S.saprophyticus. Staphylococcus-gram-positive cocci, motionless, dispute and capsules not form, in smears they are arranged in clusters in the form of "clusters of grapes". **Cultural properties** . Not demanding on nutrient media: cultivated on MPA with education pigmented colonies yellow or white colors, in BCH give diffusely turbid growth. For the identification of staphylococci, the nature of growth is important on blood agar (hemolysis zone) and yolk-salt agar (YSA) (determination lecithinase).

Biochemical properties . Staphylococci break down carbohydrates into acids. Important differentiating sign various species staphylococci is education acids from mannitol in aerobic and anaerobic conditions.

pathogenicity factors.

1. Factors adhesion:

-teichoic acids provide adhesion on cells organism;

"hospital strains" *S. epidermidis* produce a special kind of mucus that provides their attachment to polymeric materials of catheters, artificial heart valves and creation of a bacterial biofilm on them. This leads to the development of sepsis and endocarditis, conditioned "hospital strains" *S. epidermidis*.

2. Protein A non-specific binds Fc fragment IgG that leads to oppression phagocytosis, functions complement and opsonizing actions antibodies.

3. Eclipse antigens, having antigenic commonality With cells skin and kidney person.

4. Enzymes pathogenicity:

– hyaluronidase, splits hyaluronic acid in composition connective fabrics, what promotes the spread of staphylococci;

-plasma coagulase causes clotting proteins serum blood, forming fibrin

"pseudocapsule" protecting staphylococci from phagocytosis

-Plasmocoagulase is one of the important markers of various types of staphylococci for differentiation. *S. aureus* has plasmocoagulase and is coagulase-positive staphylococcus; *S. epidermidis* and *S. saprophyticus* do not have plasmocoagulase and are classified as coagulase-negative (KOS).

-fibrinolysin splits fibrin and promotes splitting staphylococci in body;

-lecithinase destroys lipid membranes cells organism;

nucleases (RNases, DNases) cleave DNA molecules and RNA, which leads to destruction synthesis squirrel in cells and their death;

- β -lactamase destroys β -lactam antibiotics (penicillins, cephalosporins).

5. Exotoxins:

- hemolysins of 4 types, mainly possessing hemolytic and cytotoxic action;

-leukocidin destroys leukocytes;

exfoliatins cause damage and detachment epidermis With accumulation liquids and formation of blisters, causing the development of the syndrome of "scalded skin" (syndrome Lyell);

-exotoxin toxic shock (EST) causes systemic defeat organism in form syndrome toxic shock (STSH) With high lethality;

Enterotoxins cause symptoms of acute food poisoning. All toxins except hemolysins, produces only *S. aureus*.

6. R-plasmids (factors multiple medicinal sustainability).

S. aureus - ubiquitous, are part of the facultative microflora of the skin and mucous shells nose and nasopharynx.

Sources infections are sick human and bacteriocarrier. Often formed carriage at medical staff.

Ways of infection: airborne, contact, alimentary. In individuals with reduced resistance available endogenous way infections.

Nosological forms of infections caused by *S. aureus* are diverse, because are amazed any fabrics and organs.

S. epidermidis colonizes the skin and mucous membranes. Most often causes nosocomial, iatrogenic infections: sepsis, endocarditis, urological infection what is associated with the colonization of artificial heart valves by these microorganisms, catheters prostheses vessels.

S. saprophyticus colonizes mucous membranes of the urogenital tract and causes inflammation various departments urinary ways at people With reduced resistance.

Main nosological forms staphylococcal infections

Forms diseases	Material for research
Local	
Purulent defeat skin (boils, carbuncles, abscesses. Phlegmon)	Purulent detachable, purulent content

mastitis	breast milk, pus from abscess
Angina, tonsillitis	Smear from pharynx, With tonsils
Pneumonia, bronchopneumonia	Sputum, flushing water bronchi, blood
Arthritis	articular liquid
Conjunctiva	Purulent detachable conjunctiva
infections urinary ways	Urine
food poisoning	Flushing water stomach, emetic masses, faeces, leftovers food
Generalized	
Sepsis	
Endocarditis	
Meningitis	
Hematogenous osteomyelitis	
Syndrome toxic shock (STSH)	Detachable from vagina, blood

specific treatment staphylococcal infections

Acute staphylococcal infections	Chronic staphylococcal infections
Immunoglobulin staphylococcal human	Anatoxin staphylococcal purified liquid
Staphylococcal bacteriophage	killed staphylococcal vaccine, chemical staphylococcal vaccines for basis protective antigens

Streptococci are Gram-positive cocci, non-motile, do not form spores or capsules. smears are located chains.

Cultural properties . streptococci demanding to nutritious Wednesdays. AT sugarbroth give near-wall type of growth. On blood agar they form small convex colonies. Optional anaerobes.

By character growth on blood agar allocate 3 groups streptococci:

- 1) α -hemolytic cells form a green zone around the colonies ("greenstreptococci") as a result transformation hemoglobin in methemoglobin;
- 2) β -hemolytic cause full lysis erythrocytes and form around colonies transparent zone;
- 3) γ -streptococci do not cause hemolysis and are non-homolytic.

Biochemical properties . When identifying streptococci, their ability to ferment carbohydrates, grow on media with bile, as well as on environments with high concentration NaCl and reduce in milk methylene blue.

Antigenic structure . By antigenic structure (polysaccharide antigens of cell walls) R. Lensfield divided streptococci on 20 serogroups - AND, AT, FROM, and etc. To streptococcus group A include - S.pyogenes (β -hemolytic - streptococcus), the most pathogenic view.

α -hemolytic streptococci are mostly part of the normal microflora ("oral streptococci", enterococci), but can cause pathology in humans when decline residency organism.

Non-hemolytic streptococci are included in composition obligate microflora mucous shells human and usually not cause pathological processes.

The most epidemiologically significant for humans is the species S.pyogenes, which has significant set **factors pathogenicity**:

1. Factors adhesion : lipoteichoic acid cellular walls;
2. Protein M provides not only adhesion, but and suppression phagocytosis;
3. Eclipse antigens having antigenic commonality with cloth hearts and kidneys.

Enzymes pathogenicity:

- hyaluronidase - promotes displacement microbes by connecting fabrics;

-fibrinolysin (streptokinase)- causes dissolution fibrin blood clots, promotes dissemination on circulatory

channel;

-DNA-aza- destroys molecules DNA.

Exotoxins :

-hemolysins (O- and S-streptolysins) - have hemolytic and cytotoxic action on cardiomyocytes and phagocytes;

-erythrogenic (pyrogenic) - lead to the formation of rashes on the skin, have pyrogenic action, cause development of the syndrome toxic shock.

A source infection : sick human and bacteriocarrier.

Ways of infection: airborne, contact, for *S. agalactiae* - intranatal (in time childbirth).

Main method microbiological diagnostics streptococcal infections is bacteriological.

PROTOCOL RESEARCH

Stage (day research)	move research	Result
1st	Microscopy swabs from pus, painted according to Gram	Among leukocytes visible Gr + cocci, located small bunches and same on alone and in pairs. colony growth medium sizes With turbidity around colonies and iridescent whisk
	Sowing in cups with bile-salt agar	
2 - th	smear microscopy from selected colonies, painted on Gramu Screening of colonies with rainbowwhisk on oblique agar	AT field vision visible Gr + cocci located forms
3rd	Identification dedicated	

	pure culture. Feature definition pathogenicity: a) smear microscopy, painted on Gram; b) inoculation on Hiss media with mannitol and glucose anaerobic and anaerobic conditions; c) definition hyaluronidase activity, plasma coagulation, DNase; d) determination of α -hemolysin on blood cups agar; e) phage typing. Sensitivity test to antibiotics method paper disks.	
4th	Conclusion research	Highlighted culture pathogenic staphylococcus. Fagotype is --- sensitive to next antibiotics

Scarlet fever is an acute infectious disease, manifested by a small punctate rash, fever, general intoxication, tonsillitis. The causative agent of the disease is group A streptococcus (*Streptococcus pyogenes*). Infection occurs from patients by airborne droplets (with cough, sneezing, conversation), a same through items everyday life (dishes, toys, underwear). Especially dangerous sick like sources infections in first days illness.

Sources pathogen infections are sick scarlet fever or any another clinical form of streptococcal infection and a bacteriocarrier. Get sick more often children aged 3-10 attending kindergartens and schools. The occurrence of cases scarlet fever in children's institutions, How rule preceded by elevated level incidence of tonsillitis and acute respiratory viral infections. Children of the first years of life (especially the first half of the year) and adults rarely get sick with scarlet fever. Basic way transmission pathogen infections — airborne.

Pathogenesis

Pathogen penetrates in organism human through mucous shells pharynx and nasopharynx, in rare cases Maybe infection through mucous shells genital bodies or damaged skin. AT place adhesion bacteria formed local inflammatory-necrotic hearth. Development infectious-toxic syndrome conditioned in first turn admission in blood flow erythrogenic toxin streptococci (toxin Dick), a same action peptidoglycan cellular walls. Toxinemia leads to a generalized expansion of small vessels in all organs, including volume including in skin covers and mucous shells, and appearance characteristic rash. Synthesis and accumulation antitoxic antibodies in dynamics infectious process, binding them toxins in subsequent condition decrease and liquidation manifestations toxicosis and gradual disappearance rash. Simultaneously develop moderate phenomena perivascular infiltration and edema dermis. Epidermis impregnated exudate, his cells exposed keratinization, what in further leads to peeling of the skin after the extinction of the scarlatinal rash. Maintaining a strong connections between keratinized cells in the thick layers of the epidermis on the palms and soles explains large-lamellar character peeling in these places.

Components cellular walls streptococcus (group A-polysaccharide, peptidoglycan, protein M) and extracellular products (streptolysins, hyaluronidase, DNA-aza and etc.) condition development reactions hypersensitivity delayed type, autoimmune reactions, formation and fixation immune complexes, violations systems hemostasis. In many cases them can think cause

development glomerulonephritis, arteritis, endocarditis and other complications of immunopathological character.

From lymphatic formations mucous shells oropharynx pathogens on lymphatic vessels enter the regional lymph nodes, where they accumulation, accompanied by development inflammatory reactions with foci of necrosis and leukocyte infiltration. Subsequent bacteremia in some cases maybe lead to the penetration of microorganisms into various organs and systems, the formation purulent-necrotic processes in them (purulent lymphadenitis, otitis, bone lesions fabrics temporal areas, solid cerebral shells, temporal sinuses and etc.).

Scarlet fever should be distinguished from measles, rubella, pseudotuberculosis, medicinal dermatitis. In rare cases, the development of fibrinous deposits, and especially when they go beyond limits tonsils disease is necessary differentiate from diphtheria.

scarlet fever distinguish bright spilled hyperemia oropharynx ("flaming yawn"), sharp limited in place transition mucous shells on solid sky, bright red language Withraspberry hue and hypertrophied papillae ("raspberry tongue"), punctate elements rashes on general hyperemic background, thickening rashes in form dark red stripes on skin folds in places natural folds, distinctly expressed white dermographism, pale nasolabial triangle (symptom Filatov). At pressure on skin palm rash in this place temporarily disappears ("symptom palms"), endothelial symptoms are positive. After the disappearance of the exanthema finely scaly peeling of the skin is noted (large-lamellar on the palms and soles). *Laboratory diagnostics*

Diagnosis scarlet fever based on clinical (acute Start diseases, fever, intoxication, acute catarrhal or catarrhal-purulent (with septic form disease - necrotic), tonsillitis, abundant point rash, thickening in natural folds skin and laboratory (neutrophilic leukocytosis, increased ESR, abundant height beta-hemolytic streptococci at sowing material from hearth infection on blood agar, increase in antibody titers to streptococcal antigens - M- protein A-polysaccharide streptolysin-O and others) data.

situational tasks :

Microscopic examination of the detachable furuncle revealed *S. aureus*. To which group representatives of normal skin microflora applies this microorganism?

On what from listed Wednesdays grow up staphylococci and kaike environments appropriate use at carrying out bacteriological research?

- a) BCH or MPA;
- b) milk-salt agar in

bilious bouillon

D) blood agar

- e) sugar agar or brothe) bile-salt agar

Which Wednesday is selective for staphylococci? What provides selectivity this environment?

What are the features of the structure of the cell wall of staphylococci in comparison with the structure cellular walls Gram-negative bacteria?

What kind pathogenicity enzymes produce staphylococci? What is their role in pathogenesis infections?

What kind cells other than erythrocytes, maybe damage ϵ - toxin?

What explains the cardiotoxic, dermatotoxic and neurotoxic effects of α -toxin?

PRACTICAL OCCUPATION No. 2.

TOPIC: PATHOGENIC DIPLOCOCCIS. MICROBIOLOGICAL DIAGNOSTICS DISEASES, CALLED PATHOGENIC streptococci And NEYSSERIES.

CHANGE MODULE ON TOPIC: "PATHOGENIC COCKIES"

TRAINING GOAL:

1. To study the morphological and cultural

properties of pathogenic gram-positive and Gram-negative streptococci and diplococci (Neisserium).

2. master main methods laboratory diagnostics diseases, called pathogenic diplococci.

STUDENT SHOULD KNOW :

1 . Morphological, cultural and biochemical features of diplococci; 2. Factors

pathogenicity, antigenic structure;

3. Sensitivity to antibiotics;

4. Main research methods: bacterioscopic, bacteriological, serological, bioassays, express diagnostics;

5. Prevention and treatment gonococcal infections.

Student should be able to :

1. Luminous microscopy drugs from pure cultures meningococci, gonococci, pneumococcus, coloring on Gram.

2. Carrying out bacteriological research: cerebrospinal fluid for suspected meningococcal infection; mucus from the patient's upper respiratory tract for pneumonia; detachable With urethra on gonorrhea (on scheme).

CONTROL QUESTIONS ON THEME LESSONS:

1. Morphological characteristic pneumococcus (Streptococcus pneumoniae), meningococcus, gonococcus.
2. Comparative characteristics of biochemical activity and the need for nutritional environments for diplococci different types.
3. Differential diagnostic signs (differences) pathogenic and non-pathogenic neisseria.
4. Factors virulence pathogenic diplococci.
5. A source infection, way transmission, input gates at diseases, caused diplococci.
6. researched material and main methods diagnostics at pathological processes, called diplococci

INDEPENDENT JOB STUDENTS

1. Studying morphology pneumococci (str. pneumoniae) in smears- prints from bodies white mice, infected intraperitoneal sputum sick pneumonia. Coloring on Gram (table).
2. The study of biochemical activity pneumococci for the purpose of differentiation them from streptococci. Sowing on environments With inulin and bile.
3. Microscopic method diagnostics acute gonorrhea: microscopy smear purulent discharge urethra of a patient with acute gonorrhea. Coloring with methylene blue.
4. Serological method diagnostics chronic gonorrhea: estimate demonstration reaction binding complement (on Borde-Jangu), delivered With goal antibody detection in serum sick gonorrhea.
5. Decor protocol research.

METHODOLOGICAL RECOMMENDATIONS

1. When a white mouse is infected with the sputum of a patient with pneumonia, the mouse dies from pneumococcal sepsis. Imprint smears are prepared from the organs of a dead mouse. paint on Gram. On pink background, educated cells fabrics, are found gram-positive diplococci slightly elongated forms, reminiscent of contours flame candles or lancet, surrounded colorless capsule.
2. A characteristic feature of pneumococci that distinguishes them from most other species of streptococci, is related to bile and bile salts. Bile not only kills, but and dissolves pneumococci. Against, in difference from verdant (S. faecalis, S. sanguis) and hemolytic streptococci (S. pyogenes), pneumococci decompose inulin.
3. Diagnosis acute gonorrhea put With help microscopic method research. From

researched material make two strokes, one color according to Gram, the other - methylene blue. If there are gonococci in the smear, they are visible gram negative diplococci, located inside leukocytes (unfinished phagocytosis).

4. So How at chronic gonorrhea gonococci are outside cells, have atypical form in form balls or very small entities, use bacterioscopic method for productions diagnosis is not possible. Therefore, for diagnostics chronic gonorrhea apply: bacteriological, serological methods research.

Serological diagnosis gonorrhea put With help RSK. Reaction put for detection antibodies in serum blood sick, With help famous antigen, which is yourself suspension killed gonococci.

Scheme productions RSK

Components reactions	1st (experience)	2nd (control AD)	3rd (control C)
1. Researched serum (1:5)	0.5	-	0.5
2. Antigen in working dose	0.5	0.5	-
3. Complement in working dose	0.5	0.5	0.5
four. Physiological solution	-	0.5	0.5
on 45 minutes in thermostat			
five. Hemolytic system	1.0	1.0	1.0
on 45 minutes in thermostat			
Accounting results		hemolysis	hemolysis

Accounting result reactions start With control tubes. At availability hemolysis incontrol test tubes about results reactions judge but experienced test tube.

INFORMATIONAL MATERIAL TO TOPIC:

Meningococci (Neisseria meningitides) are Gram-negative, bean-shaped diplococci flagella and dispute not have, in body form capsule.

cultural properties . Very demanding on cultivation conditions. grow on solid and liquid nutrient media containing 20-25% serum (serum agar, whey broth). On a dense medium they form small smooth transparent colonies. Strict temperature optimum - 37 ° C (at other temperatures, meningococci perish) must be created both during cultivation and during transportation material from sick in laboratory.

Among the representatives of the genus Neisseria there are opportunistic species, inhabitants of mucous membranes of the nasopharynx - N. Sicca, N. mucosa, etc. In people with weakened resistance, they can cause disease clinically similar with meningococcal infection.

Antigenic structure. N meningitides has generic antigens common to all species. Inside the View on capsular polysaccharide antigens distinguish between serogroups N meningitides- A,B,C,D,YZ and others

epidemiological outbreaks more often cause pathogens serogroups A,B,C.

Factors pathogenicity of meningococci :

1. Pili - provide adhesion on the cells of the cylindrical epithelium of the nasopharynx.
2. Ig A-proteases - cleave SIg A molecules, thereby reducing local protection mucous membranes of the nasopharynx;
3. Capsule - protects from phagocytosis;
4. Enzymes pathogenicity : hyaluronidase, neurominidase and others

5. Endotoxin (cell wall LPS) - causes damage to blood vessels, which appears hemorrhages in internal bodies and hemorrhagic rash on skin.

The source of infection is a sick person, or a bacteriocarrier. More often (in 70-80% cases) get sick children first three years old life.

Ways of infection - airborne. Entrance gate of infection - mucous membranes nasopharynx.

Meningococcal infection may occur in several clinical forms, who share on localized and generalized.

The main clinical forms of meningococcal infection and material for microbiological research

FORMS	DISEASES	MATERIAL FOR RESEARCH
Primary localized	meningococcal carriage	smear from nasopharynx
	Spicy nasopharyngitis	
Hematogenous generalized	Meningococemia	Smear from nasopharynx, blood
	Epidemiological cerebrospinal meningitis, meningoenzephalitis	Nasopharyngeal swab, blood, liquor

Microbiological diagnostics meningococcal infections.

1. Bacteriological method (main) - isolation of a pure culture of the pathogen on serum environments and defining its antibiotic sensitivity.
2. Bacteriological method - uses as a mandatory indicative. AT smears from native material with Gram stain, intracellular location bacteria and characteristic painting unfinished phagocytosis meningococci.

Specific prevention of meningococcal infection is carried out only according to epidemiological testimony meningococcal polysaccharide vaccine serogroups AND and FROM.

Gonococci - (*N. Gonorrhoeae*) - gram-negative bean-shaped diplococci, form capsule in body, flagella and dispute Don't Have.

Cultural properties . Demanding to nutritious Wednesdays and temperature optimum - 37 °C. Require freshly prepared moist nutrient media supplemented with native proteins blood, serum, or ascitic fluid. Does not cause hemolysis on media containing blood, on media containing with the addition of milk, gelatin and potatoes not are growing.

Gonococci are characterized by pronounced antigenic variability even within one strain.

Biochemical properties: decompose only glucose with the formation of acid. Proteolytic activity absent, ammonia, hydrogen sulfide and indole not form. **Factors pathogenicity gonococcos :**

1. Pili - provide adhesion to the cells of the cylindrical epithelium of the genitourinary ways;
2. Capsule - in freshly isolated cultures has antiphagocytic action;
3. Cellular wall contains endotoxin.
4. Surface protein 1 classes causes bactericidal factors;
5. Surface protein of class 2 forms a separate protein fraction called turbidity proteins or Opa proteins (turbidity). They are considered the first virulence factors of gonococci, and they cause attachment to epithelium.
6. R- plasmids factors multiple medicinal sustainability.

For diagnostics apply:

Bacteriological method (main) - isolation of a pure culture of the pathogen on serum media and determination of its antibiotic sensitivity. coloring by Gram and characteristic picture unfinished phagocytosis gonococci.

The serological method is used for chronic gonorrhea, in the absence of the patient's discharge. Carry out RSC on Borde-Jangu according to the standard scheme, which is positive from 3-4 weeks. Used as an antigen for RSK gonovaccine or antigen from killed gonococci.

Genetic method - determination of sections of the gonococcus genome in the material from a patient with help PCR.

For **specific treatment** chronic forms gonorrhea use killed gonococcal vaccine.

Pneumococci - Streptococcus pneumoniae Gram-positive diplococcus, usually lanceolate or arranged in chains, having a polysaccharide capsule, which allows easy "type" them specific antisera. pneumococci motionless, do not form a dispute; facultative anaerobes. When cultivating on artificial nutrient media lose the capsule, move from S - to R-shape. Good grow on blood and serum media. When grown on agar with ram's blood, they form colonies with zone α partial hemolysis and greening of the medium, β complete hemolysis, γ -hemolysis visually invisible hemolysis.

Enzymatic activity of glucose with the formation of lactic acid. Pneumococcus not contains group antigen serologically heterogeneous on AG capsule polysaccharides allocate 84 serovar.

At pneumococcal infections With goal allocation clean culture pathogen put bioassay - intraperitoneally infect white mice material from sick.

Test control

1. Explain the origin of hemolysis zones. How to distinguish pneumococci – β hemolytic - from α – hemolytic?
2. List possible methods laboratory diagnostics at gonococcal infections.
3. name main factors pathogenicity meningococcus.
4. Who is main source meningococcal infections?
5. List factors pathogenicity for meningococcus.

PRACTICAL OCCUPATION No. 3

THEME: BACTERIA - PATHOGENS INTESTINAL INFECTIONS
(pathogens intestinal escherichiosis, intestinal yersiniosis).

Learning goal: to train students in the methods of microbiological diagnostics and specific prevention intestinal diseases.

Student should know:

1. Morphology representatives intestinal groups.
2. Bacteriological method for the study of intestinal escherichiosis, intestinal yersiniosis.
3. Coloring on Gram.
4. specific prevention intestinal diseases.

The student must be able to:

1. cook and paint smear on method Gram.
2. Make a sowing of the test material on the differential diagnostic Wednesday Endo.

PLAN:

1. Taxonomy and main biological properties pathogens intestinal escherichiosis and intestinal yersiniosis.
2. Epidemiology, pathogenesis, immunity caused diseases. 3. Principles microbiological diagnostics intestinal escherichiosis and intestinal yersiniosis.
4. Preparations for etiotropic therapy and specific prophylaxis intestinal escherichiosis and intestinal yersiniosis.

INDEPENDENT JOB

Bacteriological method research

Selection clean culture from researched material (excreta patient).

1. Sowing researched material on differential- diagnostic Wednesday Endo (demonstration).
2. Accounting for the results of inoculation of the test material on the Endo medium. Selection "suspicious" colonies and their study on Endo's medium, macroscopic colony characterization (demonstration).
3. Seeding "suspicious colonies" on Ressel's medium and BCH.
4. Registration of the protocol research

PROTOCOL RESEARCH

№№ P/P	researched material	results research	Graphic image

METHODOLOGICAL RECOMMENDATIONS

AT connections With difficulty differentiation pathogens intestinal diseases, defiant similar clinical manifestations, necessary conducting integrated microbiological research, including simultaneous Search in researched material pathogens escherichiosis, shigellosis, salmonellosis and cholera.

1. The test material (stools of the patient) is inoculated on the surface of one of the differential diagnostic media for the isolation of the causative agent of intestinal diseases (Wednesday Endo) and 1 %alkaline agar for allocation pathogen cholera.

Sowing held stroke on surfaces dense nutritional environments With goal mechanical disconnect microbes and receiving isolated colonies.

Cups with 1% alkaline agar are incubated at 37 degrees. 10-12 hours, medium cups Endo - 18-24 hours.

2. After incubation in a thermostat, inoculations on plates with Endo media and 1% alkaline agar are viewed in passing and refractive light. Without any signs of microbial growth on alkaline agar, a negative answer is given for finding pathogen cholera in researched material.

On the Endo medium, after 18-24 hours of growth in a thermostat, the presence of colonies of raspberry- red (fermenting lactose incoming in composition environment) and colorless (not fermenting lactose).

3. Colorless ("suspicious") colonies are sown on Ressel's medium. Medium composition Ressel: MPA, 1 % lactose, 0.1 % glucose and indicator Andrede.

Sowing is done as follows: the removed colony carefully, without touching the edges test

tubes are introduced into the condensation liquid, then the entire beveled the surface of the medium and make an injection into the depth of the column. A test tube with seeding on Ressel's medium put in thermostat (37°C) for a day (18-24 h.).

Simultaneously for study proteolytic activity culture lactose-negative colonies sown in test tube with BCH With indicator papers, impregnated acetate lead and oxalic acid for definitions education hydrogen sulfide and indole. test tube put in thermostat (37°C, 18-24 h.)

Escherichiosis (intestinal coliinfection) is an acute intestinal infection caused by various serological groups of enteropathogenic *Escherichia coli* (EPEC), occurring with symptoms of general intoxication and a syndrome of lesions of the gastrointestinal tract.

Etiology escherichiosis.

Pathogens - enteropathogenic *Escherichia coli* - belong to the species *Escherichia*, genus *Escherichia*, family Enterobacteriaceae, are Gram-negative rods that are stable in the environment. Can be stored for months soil, water, bowel movements. Good are growing on conventional nutritional environments. Quickly perish at boiling and impact disinfectants funds. *Escherichia* have complex antigenic structure: somatic O antigen (thermostable), surface (capsular) K antigen and flagellate H-antigen (thermolabile).

Intestinal infection, caused EPEC, meet more often at children early age Classification escherichiosis:

- Enteropathogenic (salmonella-like).
- Enterotoxic (cholera-like).
- Enteroinvasive (dysentery-like).
- Enterohemorrhagic.

The diagnosis of escherichiosis can only be established by isolating the pathogen. For bacteriological research take away feces, emetic masses, flushing water stomach, at generalized forms - blood, CSF. Conduct study bowel movements are needed immediately, as soon as the patient seeks help from a doctor, since with flow time probability allocation pathogen quickly decreases. Collection bowel movements held after natural defecation or With help tampons in test tubes with a glycerin mixture in an amount of not more than 1/3 of the volume of the preservative, and emetic masses and washings of the stomach - in glass jars with a capacity of 200-250 ml. In medical the institution must conduct at least three diagnostic studies (the first - at admission sick before destination to him antibiotics, chemotherapy drugs).

In order to isolate EPEC and ETEC, stool samples should be taken from the last servings, at research EICP - samples With impurity mucus.

Selected material in flow first 2 h deliver in laboratory if this is impossible - placed in a refrigerator and sent to the laboratory no later than 12 hours after fence.

At decision question about etiological roles pathogen at occurrence intestinal infections necessary take account of the following criteria:

- isolation of *Escherichia* certain serovars related to EPEC, EIEC, ETEC, EHEC or EACPEC, in monoculture in combination with non-pathogenic *Escherichia* serovars; if *Escherichia* pathogenic diagnosis maybe to be installed by alone positive bakposeva;
- massive excretion of ETEC (10⁶/g faeces or more) and their significant predominance above representatives another conditionally pathogenic flora.

Serological research methods have a certain diagnostic value, although they are less informative, they are not convincing, since false positives are possible results due to antigenic similarities With others enterobacteria. Are used for retrospective diagnostics, especially in time outbreaks. AT the present time from serological methods research use RPHA (diagnostic titer 1:200 - 1:400 for adults, 1:40 - 1:80 for children); reaction immunofluorescence; reaction immune sorption antibodies, labeled enzymes; reaction neutralization; reaction agglutination with autoculture with an increase in antibody titer by 4 or more times in dynamics diseases.

A promising diagnostic method is the polymerase chain reaction (PCR). To prove pathogenicity *Escherichia*, need to make sure, what she It has receptors providing adhesiveness, can produce thermolabile and thermostable toxins, contains plasmid DNA,

encoding toxin formation (Protasov S.A., 2003).

If non-pathogenic *Escherichia* are isolated, it is necessary to approach the diagnosis as such at others OKI, caused conditionally pathogenic flora: triple massive height microorganism, absence sowing pathogenic pathogens.

The diagnosis of "escherichiosis", as noted, is incompetent without bacteriological, as well as serological confirmation. Exception is clinical and epidemiological justification diagnosis.

Instrumental methods surveys (sigmoidoscopy, colonoscopy) at escherichiosis uninformative.

When making the final diagnosis, the type of isolated pathogen is indicated, syndrome of lesions of the digestive tract, the severity of the disease. With a protracted the course also marks the nature of the course of the disease. For example: escherichiosis (*E. coli* O111) in form acute gastroenteritis, middle degree gravity.

Diagnosis bacteriocarrier can be installed only in those cases when clinical symptoms diseases missing in the present time and not noted in previous 1-1.5 months Bacteriocarrier, as a rule, short-term (1-2 times pathogen release). In such cases, when making a diagnosis, only the type is indicated pathogen. For example: bacteriocarrier enteropathogenic *Escherichia* O125.

Etiology. Pathogen (*Yersinia enterocolica*) - gram-negative bacillus, anaerobe, grows well on ordinary nutrient media at low temperatures. Known 30 serovars. Illness human more often cause 3rd, 5th, 8th and 9th serovars

Intestinal yersiniosis.

Epidemiology. source infections are human and animals, sick and carriers. Especially often the pathogen is found in mouse-like rodents, large horned livestock, pigs, dogs, cats, in dairy products, ice cream. Infection human going on through mouth at use infected food, water or contact way.

Disease meets during Total of the year.

Pathogenesis. Pathogen breeds in thin intestines, due to what develops enterocolitis or gastroenterocolitis. AT heavy cases in areas terminal department thin guts arises ulcerative process With involving mesenteric lymphatic nodes. At penetration pathogen in blood are celebrated bacteremia and generalization process with development inflammation in organs.

Clinic. The incubation period is 2-3 days. Clinical symptoms in patients practically does not differ from that in pseudotuberculosis. However, it is necessary to have mind that with intestinal yersiniosis, the disease often begins with intestinal disorders (copious watery stools mixed with blood), and damage to internal organs occurs How would secondarily on height of clinical manifestations and more often in heavy cases.

In the diagnosis of intestinal yersiniosis , the leading role is played by bacteriological and serological research methods. *Yersinia enterocolica* can be isolated from feces, blood, urine, pus, mucus from the pharynx, lymph node. From the methods of serological diagnostics use reaction agglutination and reaction indirect hemagglutination. Diagnostic titer 1:100 and above. More authentic increase in the titer of specific antibodies in dynamics diseases.

Prevention of intestinal yersiniosis is carried out in the same way as for other intestinal diseases. infections. specific prevention not developed.

SITUATIONAL TASKS

1. A 2-year-old child has a high temperature, diarrhea, pronounced intoxication. As necessary spend bacteriological study for productions diagnosis?
2. At carrying out bacteriological research bowel movements sick With clinical diagnosis - colienteritis, on cups with Endo medium grew colonies red color typical for *Escherichia coli*. How to solve the issue of pathogenic it intestinal sticks or No?

PRACTICAL OCCUPATION #4

THEME: BACTERIA - **PATHOGENS INTESTINAL INFECTIONS** (*pathogens abdominal typhoid, salmonellosis*).

Learning goal: to train students in the methods of microbiological diagnostics and specific

prevention intestinal diseases.

Student should know:

1. Morphology representatives intestinal groups.
2. Bacteriological method for the diagnosis of salmonellosis (food toxicoinfections).
3. Bacteriological method for diagnosing typhoid fever.
4. Bacteriological study blood cultures sick With clinical diagnosis of typhoid fever and identification selected culture according to antigenic and biochemical features. Definition of phage type and sensitivity pathogen to antibiotics.
5. Interpretation of the results of serological tests (Vidal and etc.).

The student must be able to:

1. cook and paint smear according to the method Gram.
2. sow researched material on differential diagnostic Wednesday endo, bismuth sulfite agar.
3. Spend accounting results reactions Vidal.
4. Checkout protocol research.

PLAN:

1. Taxonomy and main biological properties of pathogens abdominal typhoid, salmonellosis.
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles microbiological diagnostics abdominal typhoid, salmonellosis.
4. Preparations for etiologic therapy and specific prevention of typhoid fever, salmonellosis.

INDEPENDENT JOB

1. Accounting for the results on the differential diagnostic environment Endo, bismuth sulfite agar (demonstration).
2. Accounting for results on the Ressel medium and MPB.
3. Accounting for results reactions Vidal.

METHODOLOGICAL RECOMMENDATIONS

Abdominal typhus — acute cyclically flowing intestinal anthroponotic infection, called *Salmonella bacteria typhi* (*Salmonella enterica typhi* serotype), With alimentary through transmission (fecal-oral), characterized fever phenomena of general intoxication with the development of typhoid status, roseolous rashes on the skin, hepato- and splenomegaly and a specific lesion of the lymphatic system lower department thin intestines.

Pathogen — *Salmonella Typhi* from families Enterobacteriaceae kind *Salmonella* movable gram-negative rod with rounded ends, good paintable everyone aniline dyes. Works out endotoxin, pathogenic only for person. Not forms disputes.

Bacteria quite typhoid fever resistant in the external environment: in fresh water reservoirs, they persist for up to a month, on vegetables and fruits - up to 10 days, and in dairy products may multiply and accumulate.

Under impact 3 % solution chloramine, five % solution carbolic acid, sublimes (1:1000), 96 % ethyl alcohol they perish through some minutes.

Salmonella abdominal typhus have complex antigenic structure. Various serovars contain characteristic kit antigenic factors which add up from combinations O- and H-antigens.

laboratory diagnostics before Total is in bacteriological examination of blood, feces, urine, bile. The blood culture method can be used from the first days of illness and until the end of the febrile period, preferably before treatment. For of this, 5-10 ml of blood from the cubital vein at the

bedside of the patient is inoculated into 20% bile broth or Wednesday Rapoport, meat-peptone bouillon With 1 % glucose, or even in sterile distilled water. Volume environments — 50-100 ml. Ratio material and environments should be 1:10. Feces, urine, duodenal contents are examined from the 2nd week from the beginning diseases, sowing on environments Ploskireva, Levin, Muller and others Preliminary the result of these research receive through 2 days, final — through four day.

For identifying abdominal typhoid sticks in faeces, urine, duodenal content use REEF With labeled sera to O- and Vi antigens. Preliminary answer maybe to be received in for 1 hour, final — through 5-20 h.

Of the serological methods, RA (Vidal) and RPHA with cysteine are used. reaction Vidal is placed with H- and O-antigens from the 7th-9th day of the disease, repeated on the 3rd-4th week for determining the increase in titer (from 1:200 to 1:400-1:800-1:1600). The last one matters for exceptions positive result reactions, which maybe to be conditioned prior immunization against typhoid fever. The answer can be received through 18-20 hours. When setting up RPHA, the results are recorded after incubation of the plates at 37 ° C for 1.5-2 hours and again after 24 hours at room temperature. Positive counts reaction in titre 1:40 and higher.

Salmonellosis is an acute intestinal infection of animals and humans caused by salmonella. Acute infectious zoonanthropotic disease, called salmonella and characterized in general case, development intoxication and defeat gastrointestinal tract.

salmonellosis at human consider How certain disease (nosological form), distinguishing it from typhoid fever and paratyphoid fever. Main source infections - food products, less often - a sick animal, in some cases a source infections maybe to be human (sick or bacteriocarrier). Infection going on through contaminated food, usually of animal origin (meat and meat products, milk, eggs, especially duck and goose), at forced, wrong slaughter animals, violation rules storage and cooking products (contact finished and raw products, inadequate thermal treatment products before consumption, etc.). Salmonellosis develops when organism fall accumulated in products live salmonella.

On the territory of the Russian Federation, the following serovars of the Salmonella species are most common: enterica subspecies enterica: Salmonella enteritidis, Salmonella Typhimurium , Salmonella infantis.

Clinical manifestations salmonellosis varied — from asymptomatic carriage of the infectious agent to severe septic forms. Incubation period fluctuates from 2-6 hours before 2-3 days. There are several clinical forms of salmonellosis:

1. Gastrointestinal the form
2. Typhoid form
3. Septic the form

AT 15-17 % cases salmonellosis in period convalescence observed short-term bacteriocarrier. Possible "transient" carriage (single selection salmonella without clinical manifestations) and chronic bacteriocarrier.

Diagnostics salmonellosis carried out complex With taking into account epidemiological data, symptoms and results laboratory research, aimed at isolation and typing of the pathogen. The main type of typing salmonella is reaction agglutination. For her holding before recent time used hyperimmune sera, but now they have been replaced by monoclonal antibodies to salmonella.

Prevention.

Veterinary and sanitary supervision of slaughter and processing of carcasses; implementation of sanitary rules cooking, storage and implementation food products; examination incoming on work on enterprises public nutrition and trade, children's institutions.

SITUATIONAL TASKS

1. A patient from epid. focus of typhoid fever. Complaints: high fever, headaches, cough, abdominal pain. What laboratory method should be used to clarify diagnosis? What day will the answer be given? Positive or negative? prescription diseases 2 day.
2. From sick received hemoculture, which on morphological, cultural, biochemical properties corresponds pathogen abdominal typhus. Reaction agglutination with diagnostic serum gives a

negative reaction. Your choice? 3. How put microbiological diagnosis abdominal typhus at sick With suspicion on this is disease? prescription diseases five days.
4. What are peculiarities bacteriocarrier at abdominal typhus? What kind serological reactions are used for confirmation chronic bacteriocarrier

PRACTICAL OCCUPATION #5

THEME: BACTERIA - PATHOGENS INTESTINAL INFECTIONS (pathogens shigellosis, cholera).

Target lessons:

1. Teach students the methods of microbiological diagnostics and specific prevention intestinal diseases.

Student should know:

1. Biological properties and laboratory diagnosis of cholera. 2. Biological properties and laboratory diagnostics dysentery. 3. Express diagnostics cholera.
four. specific prevention cholera and dysentery.

The student must be able to:

1. Record and interpret the results of rapid diagnostics of cholera. 2. Carry out bacteriological diagnosis of dysentery: do sowing on differential diagnostic Wednesday Ploskirev.

PLAN:

1. Taxonomy and main biological properties pathogens of shigellosis, cholera.
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles microbiological diagnostics shigellosis, cholera.
4. Preparations for etiotropic therapy and specific prevention shigellosis, cholera.

INDEPENDENT JOB

1. Accounting results express diagnostics cholera (demonstration).
2. Accounting for the results of sowing on a differential diagnostic medium Ploskireva (macro- and microscopic research).

METHODOLOGICAL RECOMMENDATIONS

shigellosis — national team group infectious diseases, called bacteria kind shigella (Shigella).

Dysentery - shigellosis, occurring with symptoms of intoxication and predominant defeat distal department thick intestines.

Etiology.

pathogens — gram negative motionless (generic sign) bacteria kind Shigella family Enterobacteriaceae.

Epidemiology.

The source of infection is sick persons and bacteria carriers. Shigellosis is registered within Total of the year with lifting incidence in warm season.

Mechanisms transmission — fecal-oral and contact household, through water, food products. certain role in dissemination infections play insects- carriers: flies, cockroaches.

infectious dose is 200-300 alive cells, what usually enough for development diseases.

Incubation period lasts 1-7 days.

The pathogenesis of shigellosis. The entrance gate of infection is the intestine, where reproduction shigella. Invasion shigella going on predominantly in enterocytes distal department thick intestines, what leads to destruction enterocytes, development local inflammatory changes in form edema, hyperemia, erosion, superficial ulceration. Endotoxins shigella, hitting in blood, cause general intoxication, up to before development endotoxin shock violation all species exchange substances -

protein, fatty, water-salt, with development exicosis various degree.

Treatment

Etiotropic (effect on the pathogen) treatment is carried out with drugs: drugs nitrofur series (furazolidone, furadonin), quinolines (chlorquinaldone), fluoroquinolones (ciprofloxacin).

Pathogenetic treatment consists of detoxification therapy with isotonic saline solutions (solution Ringer), enterosorbents (enterosorb, Activated coal, Polyphepan, Smecta), as well as vitamin therapy. Carry out the correction of dysbacteriosis. *Laboratory diagnostics shigellosis.*

1. General blood analysis. Leukocytosis, neutrophilic shift to the left, increased ESR; degree changes usually corresponds gravity states.

2. Bacteriological method. Material for research serve excreta sick and vomit. They use differential diagnostic media (Ploskireva, Endo or Levin).

3. Serological method. Explore paired serum in RPGA With erythrocyte diagnosticum for detection antibodies and growth them titer.

Minimum conditionally diagnostic titer antibodies to diagnosticum shigella Flexner for children before 3rd years old think reaction in breeding 1:100 for the rest diagnosticums 1:200 or 4th multiple growth titra antibodies in disease dynamics.

4. Apply same immunofluorescent method, allowing discover antigen in faeces, urine, blood; reaction growth titra phage (RNF), reaction neutralization antibodies (RNA), enzyme immunoassay method (IFA) and immunoradiometric analysis (IRA).

5. Coprocytological study carry out With first days illness. At microscopic research take into account elevated number leukocytes, erythrocytes, cells intestinal epithelium, Availability starch, fat and products his splitting, cysts protozoa, eggs worms.

Cholera (IC. cholera (Greek cholera, from cholē bile + rheō to flow, expire)) - acute intestinal anthroponotic infection, called bacteria kind *Vibrio cholerae*. Characterized fecal-oral mechanism infection, defeat thin intestines, watery diarrhea vomiting, fastest loss organism liquids and electrolytes with the development of varying degrees of dehydration up to hypovolemic shock and of death.

Etiology. Known more 150 serogroups *Vibrio cholerae*; them share on agglutinating typical cholera serum O1 (*V. cholerae* O1) and on not agglutinating typical cholera serum O1 (*V. cholerae* non O1).

"Classic" cholera called cholera vibrio serogroups O1 (*Vibrio cholerae* O1). Distinguish two biovar (biotype) this serogroups: classical (*Vibrio cholerae* biovar cholerae) and El Tor (*Vibrio cholerae* biovar eltor).

By morphological, cultural and serological characteristics they similar: short curved mobile sticks, having flagellum, gram negative aerobes, Good stained aniline dyes, dispute and capsules not form, grow on alkaline media (pH 7.6-9.2) at a temperature of 10-40 °C. *Vibrio cholerae* El-Thor in difference from classical able hemolyze erythrocytes sheep (not always).

Every from these biotypes on O antigen (somatic) subdivided on serotypes. Serotype Inaba (Inaba) contains fraction C, serotype Ogawa (Ogawa) - faction B and serotype Gikoshima (more correctly Gikoshima) (Hikojima) - fractions A, B and C. H-antigen cholera vibrios (flagellate) — general for all serotypes. cholera vibrios form cholera toxin — proteinaceous enterotoxin.

Vibrio cholerae non-O1 causes cholera-like diarrhea of varying severity, which same maybe end lethal outcome

As example can lead big epidemic caused *Vibrio cholerae* serogroup O139 Bengal. She started in October 1992 in the Madras Port of South India and, rapidly spreading along the coast of Bengal, reached Bangladesh in December 1992, where only for the first 3 months 1993 caused more how 100000 cases diseases.

Laboratory diagnostics. Purpose of diagnosis: indication of *Vibrio cholerae* in stool and / or vomit, water, determination of agglutinins and vibriocidal antibodies in paired sera blood sick

Methodology diagnostics. Sowing bacteriological material (excreta, emetic mass, water) on thiosulfate-citrate-bile-salt-sucrose agar (eng. TCBS), as well as on 1 % alkaline peptone water;

subsequent reseeded on second peptone water and sowing on cups of alkaline agar.

Selection of clean culture, identification.

Study biochemical properties of the isolated culture — ability to decompose those or other carbohydrates, t. n. "row sugars" — sucrose, arabinose, mannitol.

Reaction of agglutination with specific sera

Prevention. Warning of infections from endemic foci

Compliance with sanitary and hygienic measures: water disinfection, hand washing, thermal food processing, disinfection of places of general use and t. d.

Early detection, isolation and treatment of sick and vibriocarriers

specific prevention of cholera vaccine and cholera toxin. Cholera vaccine has short (3-6 months) validity.

At the present time there are the following oral anti-cholera vaccines:

Vaccine WC/rBS — includes from killed whole cells of *V. Cholerae* O1 With purified recombinant B-subunit of cholera toxin (WC / rBS) - provides 85-90- percent protection in all age groups for six months after taking two doses With weekly break.

Modified vaccine WC/rBS — not contains recombinant B-subunits. Necessary to take two doses of this vaccine With weekly break. Vaccine licensed only in Vietnam.

Vaccine CVD 103-HgR — includes from weakened alive oral genetically modified strains of *V. Cholerae* O1 (CVD 103-HgR). Single dose of vaccine provides protection against *V. Cholerae* at a high level (95%). Three months after taking a vaccine protection from *V. Cholerae* El Tor was at the level 65 %.

SITUATIONAL TASKS

1. From bowel movements sick highlighted gr-, mobile vibrio, agglutinating about- agglutinating cholera serum, insensitive to action of a specific cholera phage, insensitive to polymyxin. Your indicative diagnosis? What need to more do for confirmation diagnosis?

2. In a patient with profuse diarrhea and vomiting and from feces and vomit allocated gr-mobile wand, not losing mobility in presence about- agglutinating cholera serum, in sowing on Poleva-Yermolyeva through 3 hours in first test tube - diffuse haze, in second - diffuse haze, in third - at adding solution Lugol turning blue. Your offer? Stages further laboratory research?

3. From water open reservoir allocated microbe: gr- wand very mobile giving on alkaline agar very delicate transparent, bluish in transmitted light colony, splitting glucose, maltose becks, not splitting lactose dulcitate, thinning gelatin funnel. Your indicative diagnosis?

PRACTICAL OCCUPATION No. 6

TOPIC: PATHOGENIC ANAEROBES. LABORATORY METHODS DIAGNOSIS, SPECIFIC PREVENTION AND THERAPIES ANAEROBIC DISEASES.

TRAINING GOAL:

1. Explore modern methods microbiological diagnostics diseases, called anaerobes.
2. To study preparations for specific prophylaxis and therapy of anaerobic diseases.

Student should know:

1. Peculiarities morphology, tinctorial and cultural properties, biochemical activity.
2. Pathogenicity factors: toxins and their significance in the pathogenesis of anaerobic infections.
3. Distribution, a source of infection, way of transmission of diseases called at person.

1. Microbiological diagnostics: bacterioscopic, bacteriological method, bioassays.

5. Specific prevention and treatment.

The student must be able to:

1. Conduct bacteriological research of clean culture (on scheme).

1. cook smear and paint on Gram.
2. Microscopy smear.
3. Spend accounting results.

CONTROL QUESTIONS

1. Modern ideas about the etiology of anaerobic infection. Clostridial and non-clostridial anaerobic infection.
2. Morphological, cultural and biochemical properties pathogens anaerobic infections: Clostridium (gas gangrene, tetanus, botulism), peptostreptococcus, bacteroids, fusobacteria, anaerobic vibrios, campylobacter and spirillum
3. Pathogenetic aspects of anaerobic infection: primary exogenous and secondary, endogenous. Mechanisms occurrence. Opportunistic anaerobic and mixed infections.
4. Methods microbiological diagnostics anaerobic infections.
5. Principles specific prevention anaerobic infections. Preparations for active and passive immunization.
6. Principles specific therapy anaerobic infections. Etiotropic and pathogenetic therapy: antibacterial, hyperbaric oxygenation and etc.

INDEPENDENT JOB

1. Microscopic diagnostic method gas gangrene: study of smear-imprint from purulent wounds, coloring by Gram.
2. Bacteriological method diagnostics anaerobic infections:
1-th stage - studying on five% bloody agar isolated colonies bacteroids and peptostreptococcus, allocated from purulent exudate.
Further- receiving clean culture anaerobic bacteria in semi-liquid environment AS. Demonstration of selective anaerobe culture media: Kitta-Tarozzi, "high"column sugar agar.
2-th stage - identification of a pure culture of anaerobic bacteria by biochemical properties With using test systems AP1-Ap (principle "variegated row").
3. Determination of sensitivity of anaerobic bacteria to antibiotics (micromethod). Demonstration of results sowing clean culture into a microcassette With antibiotics.
4. Description of drugs for the specific prevention of clostridial anaerobic infections: tetraanotoxin gas gangrene, pentaanotoxin (+ tetanus toxoid), tetanus toxoid component drugs ADS and vaccines DPT, TABte.
5. Description drugs for specific therapy clostridial anaerobic infections: polyvalent antigangrenous serum, antitoxic tetanus toxoid, antitoxic monoclonal and polyvalent anti-botulinum serums.
6. Decor protocol research.

METHODOLOGICAL RECOMMENDATIONS

1. Microscopic method diagnostics gas gangrene. AT smear- imprint from purulent wounds (coloring on Gram) are found rod-shaped cells purple colors.
2. Bacteriological method diagnostics anaerobic infections.
1-th stage. First day. On 5% blood agar in a Petri dish (after cultivation in anaerostat: 80% N₂ , _ 10% H₂ _ 10% CO₂) determined some species isolated colonies, including those with various types of hemolysis (α , β) and pigment (for example, black pigment bacteroids groups melaninogenicus). **Second day.** AT test tube With clean culture peptostreptococci in semi-liquid environment AC observed small granules white colors at the bottom parts of the test tube with the medium. When monitoring the purity of the isolated culture (coloring gentian violet) chains are defined from elongated cocci of blue color.
2-th stage. AT test system API-An for identification pure cultures on biochemical properties determined fermentation glucose (change coloring indicator in yellow) in the absence of other manifestations of glycolytic, as well as proteolytic activity (negative samples on indole and

hydrogen sulfide).

3-th stage. At definition sensitivity anaerobic bacteria to antibiotics in microcassette (after cultivation in anaerostC) are celebrated positive and negative options results.

4- stage . At studying ampoules With drugs for specific prevention and therapy anaerobic infections, in protocol are celebrated goals (prevention, treatment), nature of immunization (active or passive, antitoxic or antibacterial), testimony to application and peculiarities use everyone drug.

PROTOCOL RESEARCH

№№ P/P	researched material	results research	Graphic image
1.	Smear-imprint from a festering wound. Coloring on Gram.		

Informational material

Tetanus severe wound infection.

Morphology Gram-positive rods with rounded ends. located alone or chain. controversy situated terminal.

Cultural properties obligate anaerobe. On MPA and gelatin in strictly anaerobic conditions pathogen growing slowly and forms thin transparent colonies. When sowing column in semi-liquid agar through 24-48 hours forms colonies in form "lentils" R –forms or "fluff" S forms.

Pathogenicity factors are exotoxins tetanospasmin and tetanolysin.

Antigenic structure – Oh and H antigens.

Immunity. There is no natural immunity in humans to tetanus. Diagnostics:

bacterioscopic, bacteriological and biological.

Treatment sent on neutralization tetanus toxin toxoid. Apply tetanus toxoid horse serum in dose 50-100 thousand ME.

Prevention - surgical treatment of the wound. Creation of an artificial active immunity in a planned manner, vaccination of DTP, ADSm. Primary vaccination carry out children in 3-monthly age.

Clostridia botulism

Botulism is an acute food poisoning that occurs predominantly defeat central and vegetative systems.

Morphology - rods with rounded ends, movable, peritrichous. controversy situated subterminally.

Cultural properties - strict anaerobes. Grow well on Kitta-Tarozzi media, bouillon from meat fish. calls turbidity environments and gas formation.

Everybody types clostridia botulism form hydrogen sulfide.

The antigenic structure has group-specific (H) flagella and type-specific somatic (O) antigens.

Pathogenicity factors - botulinum toxin is a protein that exhibits neurotoxic action. Botulinum toxin is most strong poison, famous to a person.

Immunity. Natural immunity human absent.

Treatment. For treatment on Bezredko sick i/v introduce one international medical dose (on

10000 IU serum types AND and E and 5000 IU type C).

Prevention. For emergency prevention used polyvalent (types A, B, E) equine serum.

Clostridia gas gangrene.

Anaerobic wound infection (gas gangrene, anaerobic myositis) - severe wound infection human infection and animals, called bacilli kind *Clostridium perfringens*.

Morphology. Vegetative cells - large, gram-positive, motionless.

Classic forms submitted under direct corner ends. AT body form capsules, they are the most expressed in virulent strains. Resistant to phagocytosis.

cultural properties. On dense media, *C. Perfringens* of type A forms S and R - round colonies. S - dome-shaped colonies, with smooth even edges. R - colonies wrong forms edges; in depth of agar resemble lumps cotton wool.

Growth on liquid and semi-liquid nutrient media, especially those containing glucose, very rapidly with the formation H₂ and CO₂ and usually ends in 8-12 hours. Turbidity of the medium and active gas formation can be observed after 4-8 hours cultivation.

Biochemical activity- splits With education acids and gas glucose, sucrose, maltose lactose mannose starch.

Proteolytic activity weak; liquefies gelatin, intensively curdle milk.

Antigenic structure - Everybody serovars form α - toxin (lecithinase). Pathogen forms at least 12 identifying toxins and enzymes that play a role in pathogenesis gas gangrene.

Clostridium perfringens is widely distributed in the environment; it is isolated from water, soil, sewage. Spores can persist in the environment for a long time. environment, able to vegetate in the soil. Disputes distinguishes high resistance to chemicals and physical influences.

situational tasks

1. Reply on test question: select environment, on which cultivated clostridia: a) iron sulfite milk

b) a high column of sugar MPA in)

Wednesday Endo, Levina

d) Wilson-Blair

environment e) bilious

bouillon

e) blood agar

2. What kind methods laboratory diagnostics You you can Mark for gas gangrene and tetanus, proceeding from from knowledge pathogenesis, clinical picture and infection conditions?

3. It is important to know that in the pathogenesis of diseases caused by gas gangrene and tetanus, main role belongs produced them toxins and enzymes pathogenicity.

4. name them, give brief characteristics them properties.

5. Taking into account this fact, suggest drugs for a specific prevention and treatment of anaerobic infections caused by gas gangrene and tetanus

PRACTICAL OCCUPATION #7

TOPIC: **DIAGNOSTICS OF ZONONOSE** **BACTERIAL** **INFECTIONS**
(tularemia brucellosis)

Educational goal:

1. Teach students the methods of microbiological diagnostics and specific prevention brucellosis, tularemia.

Student should know:

1. Biological properties and laboratory diagnostics brucellosis, tularemia.
2. specific prevention brucellosis, tularemia.

The student must be able to:

1. Record and interpret results the Hedderson reactions and Wright at brucellosis.
2. Spend accounting and interpretation results reactions agglutination at tularemia.

PLAN:

1. Taxonomy and main biological properties of pathogens brucellosis, tularemia.
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles microbiological diagnostics brucellosis, tularemia
4. Preparations for etiotropic therapy and specific prevention brucellosis.tularemia
5. Sacha module.

INDEPENDENT JOB

1. Setting and taking into account the results of the Hedderson reaction and Wright at brucellosis.
2. staging and accounting results reactions agglutination at tularemia

**GUIDELINES SEROLOGICAL DIAGNOSIS
brucellosis**

Serum of patients with brucellosis accumulates agglutinating (initially Ig M, then Ig D), incomplete blockers (Ig A , IgD) and opsonic (Ig D) antibodies. For their detection for diagnostic purposes using the reaction of Wright and Hedelson. Reaction agglutination- one from major diagnostic methods at brucellosis.

1. staging reactions Wright held With goal definitions content in blood serum sick specific antibodies. Components reactions:
 - a) researched serum in breeding 1:25;
 - b) antigen — suspension killed brucella (diagnosticum Wright).

SCHEME REACTIONS WRIGHT.

No. of tubes	1	2	3	four	five	6	7
Components							
1. Physiological solution	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2. Serumsick (1:25)	0.5	0.5	0.5	0.5	0.5	-	0.5
3. Breeding serum	1:50	1:100	1: 200	1:400	1:800	-	-
4. Diagnosticum Wright	0.5	0.5	0.5	0.5	0.5	-	-

Accounting results held through 18-20 hours, so on lesson offered demonstration reactions Wright. students carry out results accounting and make a conclusion.

2. staging reactions Hedderson.

Reaction put at mass survey on brucellosis With using glass plates. Components reactions:

- undiluted serum blood sick;
- antigen - suspension killed and painted crystal violet brucella.

SCHEME REACTIONS HEDDELSON

number of squares	1	2	3	four	Control	
Components					serum	antigen
1. Physiological solution	-	-			0.03	0.03
2. Serumsick	0.08	0.04	0.02	0.01	0.02	-
3. Diagnosticum Wright	0.03	0.03	0.03	0.03	-	0.03

Students conduct the reaction on their own and makes a conclusion.

CONCLUSION:

PROTOCOL RESEARCH

No.№ P/P	researched material	results Research	Graphic image

Brucellosis (IC. brucellosis) — zoonotic infection, transmitted from sick animals man, characterized multiple defeat bodies and systems organism person.

Pathogen diseases — group microorganisms kind brucella. pathogenic for There are three human beings: the causative agent of brucellosis in small cattle (*Brucella melitensis*), the causative agent of brucellosis in cattle (*Brucella abortus*), the causative agent of brucellosis pigs (*Brucella suis*).

pathogens brucellosis — bacteria kind brucella — Good tolerate low temperatures and freezing, they remain in water for up to 5 months, in soil - 3 months. and more, in cow's milk - up to 45 days, in cheese - up to 60 days, in butter, cream, yogurt and fresh cheeses — in flow Total period them food values; in frozen meat — St.

five month, in saline skins — 2 month, in wool — before 3-4 months At boiling and pasteurized milk brucella die. Disinfectants kill bacteria in for several minutes.

Most often brucellosis get sick homemade animals (goats, sheep, cows, pigs), while in animals abortions and the birth of a dead fetus are observed. *Brucella* excreted into the environment with milk, urine of sick animals and uterine discharge (in time abortion). causative agents of brucellosis same contained in meat sick animals.

AT organism human brucella penetrate through mucous shells digestive and respiratory tract, a same through damaged skin (abrasions, scratches). A person becomes infected with brucellosis by drinking raw milk from sick people. animals and dairy products prepared from it (cheese, butter, cottage cheese, cheese), and also not enough boiled and fried meC. Infection can also occur in production, related With processing skin and wool, a same at care per sick animals and through objects contaminated with their secretions. Milkmaids are most often ill, calves, shepherds, shepherds, vet. workers, zootechnics.

Incubation period (hidden) continues from one weeks before several months, usually 1-3 weeks. brucellosis is characterized by a variety of clinical symptoms; its course can be of varying severity. The disease begins gradually: appear malaise, insomnia, sometimes irritability, head pain, pain in muscles and joints, declining appetite, temperature rises before 37.1-37.3°. More often brucellosis starts acute: temperature rises before 39— 40° appear chills, weakness, plentiful sweating, sharp pain in muscles stiffness and pain in joints. Characteristically defeat circulatory vessels, nervous systems and boneus- tavnogo apparatus, sometimes there may be mental disorders. The illness lasts an average of 3 month, but maybe drag on before 1-2 years old and more. Persistent residual phenomena after transferred brucellosis may lead to disability. At pregnant women women at brucellosis possible spontaneous miscarriage.

Laboratory the confirmation brucellosis significantly limited topics what *Brucella* are dangerous pathogens, the isolation of which can only be carried out in special laboratories equipped in accordance with the requirements of prevention. In serological and allergological studies, it must be taken into account that in vaccinated vs brucellosis (grafted groups risk, professionally contacting With animals) may to be and enough prolonged time positive results How serological reactions, so especially allergic samples

From serological reactions most informative is reaction agglutination (reaction Wright). Agglutination on glass (reaction Huddleson) for diagnostics not is used, it is proposed to identify individuals to be screened for brucellosis, at massive surveys on epidemiological testimony. Reaction Heddleson often gives false positive results. AT some degree this is related With cross reactions With nearby antigens (yersinia, pathogen tularemia, anti-cholera vaccination and etc.). Should take account of, what *Br. melitensis* and *Br. abortus* cross-react with each other but not with *Br. canis*, so to detect antibodies to this brucella needed special diagnosticum, which bye more not is issued.

Perhaps, this is one from reasons rare identifying given varieties brucellosis.

At acute septic form brucellosis antibodies start come to light on 2nd week diseases and in the future their titer increases. Allergy test becomes positive in end 1st and on 2nd week. At chronic growth patterns titra antibodies often fails to identify. It should be borne in mind that the setting of an allergic test (Burne test) maybe drive to the appearance of antibodies or to an increase in titer. Other serological reactions (RSK, RPGA, OFR) less informative on compared to the reaction Wright and not have significant values. Negative results samples Burne allow exclude brucellosis (per exception HIV-infected at which disappear Everybody reactions HRT).

SITUATIONAL TASKS

1. What methods of laboratory diagnostics can you note for brucellosis, based on knowledge pathogenesis, clinical paintings and conditions infections?
2. What kind methods laboratory diagnostics You you can Mark for tularemia, proceeding from from knowledge pathogenesis, clinical paintings and conditions infections?

PRACTICAL OCCUPATION #8

THEME: BACTERIA - PATHOGENS CONTACT INFECTIONS
(pathogens Siberian ulcers and plague).

Educational goal:

1. Teach students the methods of microbiological diagnostics and specific Siberian ulcers and plague.

Student should know:

1. Biological properties and laboratory diagnostics Siberian ulcers and plague.
2. specific prevention Siberian ulcers and plague.

The student must be able to:

1. Put reaction thermoring precipitation on Askoli.
2. cook smear and paint over method Gram.

PLAN:

1. Taxonomy and main biological properties pathogen Siberian ulcers and plague
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles microbiological diagnostics Siberian ulcers and plague.
4. Preparations for etiotropic therapy and specific prevention Siberian ulcers and plague.

INDEPENDENT JOB

1. staging reactions thermoring precipitation on Askoli.
2. Accounting reactions RP on Ascoli and do conclusion.
3. Demo smear from autoclaved pus carbuncle from the patient
Siberian ulcer. coloring by Gram.

METHODOLOGICAL RECOMMENDATIONS

Anthrax bacilli are very large (6-10 microns) Gram-positive rods. with chopped ends, in a smear from pure culture are located in short chains (streptobacilli). motionless, form located centrally controversy, a same capsules.

Cultural properties: Anthrax bacilli are aerobes. grow well on simple nutrient media, at a temperature of 12-45 C. On liquid media, they give near-bottom growth in form lump cotton wool; on dense media form large, With uneven edges, rough matte colonies under magnifying glass colonies remind mane lion or head jellyfish. On environments containing 0.05- 0.5 U/ml penicillin, through 3-6 h growth anthrax bacilli form spheroplasts, located chain and reminiscent of in smear pearl necklace .

Biochemical properties: Ferments to acid glucose, saccharose, maltose, starch, inulin; have proteolytic and lipolytic activity. Highlights gelatinase, show low hemolytic, lecithinase and phosphatase activity.

Antigens and factors pathogenicity: Contain generic somatic polysaccharide and specific protein capsular antigens. Produce protein exotoxin possessing antigenic properties and consisting of several components (lethal, protective and edema factor). pathogenic for human and many animals.

Resistance: The vegetative form is unstable to environmental factors, but is extremely resistant and stored in environment decades, withstand boiling and autoclaving. anthrax bacilli sensitive to penicillin and other antibiotics; spores are resistant to antiseptics and disinfectants. Sporocidal effect possess activated solutions chloramine, hot formaldehyde, peroxides hydrogen.

Epidemiology and pathogenesis: A source infections - sick animals. More often cattle: sheep, goats, horses, deer, buffaloes, camels, pigs. Person is biological a dead end. For Siberian ulcers characteristic multiplicity mechanisms, ways and factors of transmission. A person becomes infected mainly by contact, less often alimentary, aerogenic, etc. when caring for sick animals, slaughter, processing animal raw materials, use meat and others livestock products. Susceptibility to the pathogen relatively low.

The entry gates of infection in most cases are damaged skin, much less often the mucous membranes of the respiratory tract and the gastrointestinal tract. AT pathogenesis is based on the action of the exotoxin of the pathogen, some fractions of which cause protein coagulation, tissue edema, lead to the development of infectious-toxic shock.

Clinical picture: There are skin, pulmonary and intestinal forms of Siberian ulcers. At skin form on place implementation pathogen appears characteristic anthrax carbuncle (hemorrhagic-necrotic inflammation of the deep layers of the skin with skin necrosis and the formation of a brown-black crust), this form is accompanied by edema. Pulmonary and intestinal forms relate to generalized forms and expressed hemorrhagic and necrotic defeat relevant organs.

Duration incubation period - from several hours before 8 days in average 2-3 days. Generalized forms in one hundred% cases end fatally.

Microbiological diagnostics: Material for research serve content carbuncle, sputum, cal, blood, urine. Microbiological diagnostics carried out in compliance with safety regulations, as in especially dangerous infections. For diagnostics apply Everybody five methods microbiological diagnostics. smears stain by gram, and to detect capsules - according to Ramanovsky-Giemsa, dispute - according to Ozheshka. For allocation clean culture researched material sow on meat-peptone agar and meat-peptone bouillon, a same infect laboratory animals (white mice maritime pigs). Dedicated clean culture identify on generally accepted scheme, taking into account morphology, the nature of growth on MPA and BCH, biochemical and cultural properties. Anthrax antigens define in REEF and reactions thermoprecipitation according to Ascoli also examine animal corpses, skin and products from it, skins, fur, wool and others products from animal raw materials.

Treatment: Apply antibiotics and anthrax immunoglobulin.

Prevention: For specific prevention use live anthrax vaccine STI (sanitary institute). For emergency prevention appoint anthrax immunoglobulin.

Plague (IC. pestis - infection) - an acute natural focal infectious disease group of quarantine infections, proceeding with an exceptionally severe general condition, fever defeat lymph nodes, lungs and others internal bodies, often With development of sepsis. The disease is characterized by high mortality and extremely high infectiousness.

Plague wand (IC. *Yersinia pestis*) is a bacterium discovered in 1894 at the same time two scientists: Frenchman Alexandre Yersin and Japanese Kitasato Shibasaburo.

Incubation period lasts from several hours before 3-6 days. Most The most common forms of plague are bubonic and pneumonic. Mortality in bubonic form plague reached 95%, with pulmonary - 98-99%. Currently with proper treatment mortality is 5-10 %.

The well-known plague epidemics, which claimed millions of lives, left a deep mark on stories humanity.

The causative agent of plague is resistant to low temperatures, well preserved in sputum, but at a temperature of 55 ° C dies within 10-15 minutes, and when boiled - almost immediately. Enters the body through the skin (with a flea bite, usually *Xenopsylla cheopis*), mucous shells respiratory ways, digestive tract, conjunctiva.

By main carrier natural foci plague subdivide on ground squirrels, marmots, gerbils, voles and pikas. In addition to wild rodents, in the epizootic the process sometimes includes the so-called synanthropic rodents (in particular, rats and murine), as well as some wild animals (hares, foxes), being the object hunting. From domestic animals plague get sick camels.

At natural hearth infection usually going on through bite fleas, previously fed on sick rodent. At bite infected plague bacteria fleas at human on place bite maybe arise papule or pustule, filled hemorrhagic content (skin the form). Then process spreads on lymphatic vessels without manifestation of lymphangitis. Reproduction of bacteria in macrophages lymphatic nodes leads to them sharp increase, merger and education conglomerate (bubonic form). Further generalization of an infection that is not strictly mandatory, especially in the context of modern antibiotic therapy, may lead to the development of a septic form, accompanied by a lesion practically all internal organs. However With epidemiological positions the most important role play

"screenings" of infection in the lung tissue with the development of the pulmonary form of the disease. Since development of plague pneumonia, a sick person himself becomes a source of infection, but with In this case, the pulmonary form of the disease is already transmitted from person to person - an extremely dangerous one, with very fast flow.

Establishing an accurate diagnosis must be carried out using bacteriological research. The material for them is a punctate of a festering lymph node, sputum, blood sick, detachable fistula and ulcers.

laboratory diagnostics carried out With help fluorescent specific antisera, which stain smears detachable ulcers, punctate lymphatic nodes, culture, received on the blood agar.

First vaccine vs plague created in early XX century Vladimir Khavkin. The treatment of plague patients is currently reduced to the use of antibiotics, sulfonamides and therapeutic anti-plague serum. Prevention of possible foci disease is to carry out special quarantine measures in port cities, deratization all ships, which walk international flights, creation special anti-plague institutions in steppe areas, where found rodents, detection of plague epizootics among rodents and their control. Disease outbreaks so far since meet in some countries Asia, Africa and South America.

TEST TASKS

1. reaction thermoprecipitation usually use for search anthrax antigen in:

- A. urine
- B. Bowel movements
- C. Liquor
- D. Wool and skins animals

2. Nutrient media for the cultivation of the anthrax pathogen:

- A. JSA
- B. blood agar
- C. Alkaline agar
- D. MPA

3. Morphological and tinctorial properties anthrax bacilli:

- A. Gram positive streptobacilli
- B. Form a capsule
- C. form spores
- D. mobile

4. Factors pathogenicity anthrax bacilli:

- A. drinking
- B. controversy
- C. Endotoxin
- D. Exotoxin

5. Test "pearl necklaces» on environment With penicillin apply for identification:

- A. Yersinia
- B. Francisella
- C. Brucella
- D. Anthrax bacilli

PRACTICAL OCCUPATION #9

THEME: Diagnosis of rickettsiosis, chlamydia, mycoplasmosis, ehrlichiosis.

Educational goal:

1. Train students methods microbiological diagnostics and specific prevention loose typhoid, chlamydia, mycoplasmoses, ehrlichiosis

Student should know:

1. Biological properties and laboratory diagnostics typhus, chlamydia, mycoplasmoses, ehrlichiosis
2. Specific prevention loose typhoid, chlamydia, mycoplasmoses, erlichioses.

Student should be able to:

1. Put reaction binding complement at loose typhus.
2. Put reaction agglutination at loose typhus.
3. Put reaction indirect hemagglutination at loose typhus.
4. Put reaction immunofluorescence at loose typhus.

PLAN:

1. Taxonomy and main biological properties of causative agents typhoid, **chlamydia, mycoplasmoses, ehrlichiosis**
2. Epidemiology, pathogenesis, immunity called diseases. Principles of microbiological diagnosis of typhus, **chlamydia, mycoplasmoses, ehrlichiosis**
3. Preparations for etiologic therapy and specific rash prevention typhoid, chlamydia, mycoplasmoses, ehrlichiosis

INDEPENDENT JOB.

1. Staging reactions binding complement at loose typhus With two antigens (from Rickettsia Provaschek and Muser). Accounting for results RSK (demonstration).
2. Staging reaction agglutination in typhus (Weyl reaction-Felix-K proteus OH19). Accounting results RA (demonstration).
3. Staging RPHA with rash typhus (With erythrocyte antigen).
4. Staging RPHA With erythrocyte diagnosticum at plague. Accounting results RPHA (demonstration).
5. Staging reaction immunofluorescence (demonstration).
6. Microscopy of finished preparations with the causative agent of **chlamydia, mycoplasmosis, ehrlichiosis** and rickettsia.

METHODOLOGICAL RECOMMENDATIONS

Typhus is a group of infectious diseases caused by rickettsiae acute infectious disease transmitted from a sick person to a healthy person through lice.

Epidemiology. At present, the high incidence of typhus has persisted only in some developing countries. However, the long-term preservation of rickettsia in previously ill with typhus and the periodic appearance of relapses in the form of a disease Brill-Zinsser not Maybe at deterioration social conditions (increased migration population, pediculosis, malnutrition and etc.).

source infections is sick human, beginning With recent 2-3 days incubation period and up to the 7-8th day from the moment of normalization of body temperature. After this, although rickettsia may for a long time persist in body, convalescent already danger for surrounding not is. Synonym

typhus transmitted through lice, mainly through clothes, less often through head ones. After feeding on the patient's blood louse becomes contagious through 5-6 days and before end life (then eat 30-40 days). Infection human going on through rubbing faeces lice in damage skin (in combinD). known cases infections at transfusion blood, taken at donors in last days of incubation period. Rickettsia circulating in North America (R. canada), transmitted ticks.

Endemic loose typhus

Endemic typhus (rat, flea, or American typhus) caused by rickettsiae *R. mooseri*. in the US every year registered about 40 cases diseases. It meets in regions With relatively warm climate in both hemispheres predominantly in summer and in mostly among rural residents; leaks easier, how epidemic typhoid. it disease main way rats, which transmitted to humans when bitten by rat fleas. Therefore, the fight against rats is extremely important as preventive measure.

Epidemic loose typhus

Epidemic loose typhus, known same How classical, European or lousy loose typhus, shipboard or prison fever, called rickettsiae *Provachek*.

The causative agent is a Gram-negative small non-motile bacterium *Rickettsia prowazeki*. It does not form spores and capsules, it is morphologically polymorphic: it can look like cocci, rods; all forms remain pathogenic. Usually they are stained according to the Romanovsky-Giemsa method. or silver plating according to Morozov. Cultivated on complex nutrient media, in chicken embryos, in the lungs of white mice. Reproduce only in the cytoplasm and never in the nucleus infected cells. Possess somatic thermostable and type-specific thermolabile antigen, contain hemolysins and endotoxins. in lice excrement, falling on clothes, saves viability and pathogenicity in flow 3 months and more. At temperature 56 °C dies per 10 min, at one hundred °C - per thirty With. Quickly inactivated by the action of chloramine, formalin, lysol, acids, alkalis in ordinary concentrations. Referred to second pathogenic group.

Pathogenesis.

The gates of infection are minor skin lesions (usually scratchinD), after 5-15 min rickettsia penetrate into the blood. Reproduction of rickettsia occurs intracellularly in endothelium vessels. it leads to swelling and desquamation endothelial cells. Caught in current blood cells are being destroyed released at this rickettsia infect new endothelial cells. The most rapidly breeding process of rickettsia happening in the last days incubation period and in first days fever.

Diagnosis sporadic cases in elementary period disease (before appearance typical exanthema) very difficult. Serological reactions become positive same only from 4-7 days from the onset of the disease. Easier diagnosis during outbreaks epidemiological data (intelligence about incidence, availability lice, contact with patients with typhus, etc.). When an exanthema appears (that is, from the 4th-6th day disease), a clinical diagnosis is already possible. The timing of the appearance and nature of the rash, hyperemia face, enanthema Rosenberg, spots Chiari-Avtsyna, changes co sides nervous systems

- all this allows you to differentiate primarily from typhoid fever (gradual Start, lethargy sick, changes co sides bodies digestion, more late appearance of exanthema in the form of a roseolopapular monomorphic rash, the absence petechiae and etc.).

It is necessary to differentiate from other infectious diseases that occur with exanthema, in particular, with other rickettsiosis (endemic typhus, tick-borne rickettsiosis of North Asia, etc.). Some differential diagnostic value has a blood picture. Typhoid fever is characterized by moderate neutrophilic leukocytosis with stab shift, eosinopenia and lymphopenia, moderate increase ESR.

For confirmation diagnosis use various serological reactions. Retained some value Weyl-Felix reaction - agglutination reaction with *Proteus* OXig, especially with an increase in antibody titer during the course of the disease. The most commonly used RSC rickettsial antigen (cooked from rickettsia *Provachek*), diagnostic titer is considered to be 1:160 and above, as well as an increase in antibody titer. Others also use serological reactions (reaction microagglutination, hemagglutination and etc.).

In the memorandum of the WHO meeting on rickettsiosis (1993), as a recommended diagnostic procedures recommended indirect reaction immunofluorescence. AT sharp phase disease (and period convalescence) antibodies connected With IgM, what used to distinguish from

antibodies as a result of a previous illness. Antibodies begin to be detected in the blood serum from the 4-7th day from the onset of the disease, the maximum the titer is reached after 4-6 weeks from the onset of the disease, then the titers slowly decrease. After suffering typhus, *Provisionally* persist for many years. in the body of a convalescent, this leads to a long-term preservation of antibodies (associated with IgG same in flow many years old, although and in low credits). AT last thing time With diagnostic purposes use trial therapy with tetracycline antibiotics groups. If, when prescribing tetracycline (at usual therapeutic doses), after 24-48h does not occur normalization of body temperature, it allows to exclude typhus (if fever not related With any complication).

Main *etiologic drug* in the present time are antibiotics tetracycline group, with intolerance to them, levomycetin also turns out to be effective(chloramphenicol).

For *prevention* loose typhus big meaning It has struggle co lice,early diagnostics, insulation and hospitalization sick loose typhus, needed careful sanitization of patients receptionist peace of the hospital and pest control clothes sick. For *specific prevention* used inactivated formalin vaccine containing killed *Rickettsia* *Provisionally*. Vaccines have been used intime of increased incidence and were effective. Currently available active insecticides, effective methods etiologic therapy and low incidence meaning anti-typhoid vaccination much decreased.

Cause **urogenital chlamydia** are chlamydia - gram negative bacteria, which lost some important mechanisms workings metabolic energy. This defect conditions their intracellular height, thanks to whom they have access to energy-rich metabolic intermediates. They are divided into two species - *Chlamydia trachomatis*, which combines human pathogens, and *Chlamydia psittaci*, which includes related microorganisms that primarily affect mammals and birds. Together they form genus *Chlamydia*, representatives whom possess bacteria-like morphological characteristics and unique cycle development.

Chlamydia in process reproductions undergo row successive changes. infectious particle is yourself small cage (elementary corpuscle)diameter about 0.3 micron With electron dense nucleoid. This particle penetrates in host cell during phagocytosis. From the surface membranes of the cell host around this small particles formed vacuole. Petite particle turns in large (reticular corpuscle), diameter 0.5-1.0 micron, which deprived electron dense nucleoid. Inside educated membrane vacuoles large particle increases andrepeatedly divided by formation of a transverse septum. In the end account all vacuole is filled small particles, formed from major calf at them transverse division, and turns in "inclusion" in cytoplasm cells owner. newly formed small particles may go out from cells host and infect new cells. Cycle breeding chlamydia implemented at them interaction With sensitive cell and takes 24-48 h.

Chlamydial infection at men and women most often It has incubation period from 5-7 before thirty days. She maybe call various pathologies.

In men, the urethra is primarily affected, and then other organs. (prostate gland, seminal vesicles, appendages). Canal is more commonly affected in women cervix, after which an ascending infection may occur that captures the uterus, the fallopian tubes, ovaries, a same peritoneum.

Chlamydia not are representatives normal microflora person. Them detection indicates an infectious process, and the absence of clinical symptoms diseases defines only temporary equilibrium between parasite and host in conditions that limit the reproduction of a pathogenic intracellular microorganism, butnot obstructing to him.

Clinically asymptomatic chlamydial infection not less dangerous how her manifest forms, and requires medical and preventive events.

Various methods are used **to detect chlamydial infection , such as direct** definitions pathogen, So and indirect serological examinations.

The material for research in urogenital chlamydia are smears, scrapings from the mucous membrane of the urethra, cervical canal, cervix, rectum, conjunctiva, which take away special spoon special tampons brushes or platinum loop. The collection of material is the most important step. diagnostics. When testing for chlamydia by culture, patients should not use antibiotics and other drugs active against chlamydia for a month. If are used cytological methods, drugs it is forbidden apply per 2 weeks before research.

cultural method identifying chlamydia - "gold standard" - is most informative (100% sensitivity), but due to the high cost and complexity is widespread. This method is very important if long-term infection.

Cytological method is in microscopic research superficial scrapings epithelial cells, taken from urethra, cervical channel and others mucous shells. With goal discover chlamydia. AT cooked smears, which predominantly stain, determine the presence of specific cells in cellular elements chlamydial inclusions. These intracellular inclusions are more often detected with fresh and untreated infection. The method is simple, accessible, but not sensitive enough; allows the diagnosis of chlamydia infection not more than at 15-20% sick.

Immunofluorescent method - staining chlamydial antigens immunofluorescent dyes on basis monoclonal antibodies. His disadvantage is subjectivity estimates results.

The polymerase chain reaction (PCR) method in the diagnosis of chlamydial infection is method definitions specific site DNA. With help DNA analyzer. He has very high sensitivity and specificity.

Serological method identifying chlamydia - detection antichlamydial antibodies in blood. In acute infection diagnostic value is the detection of chlamydial immunoglobulin M (IgM) -antibodies or 4-fold increase in immunoglobulin G titers (IgD) in dynamics after 2 weeks. Medium and low titers of antibodies to chlamydia, as a rule, characteristic of chlamydial cells absorbed by *Trichomonas vaginalis* (during treatment destruction of the trichomonas cell occurs and a new one enters the extracellular space portions of chlamydia, which, in turn, stimulate the production of antibodies in the body). It is forbidden. With confidence declare about infected chlamydia only on basis availability antichlamydial antibodies. Only combination various methods (not less 2 simultaneously and one from them PCR) gives necessary accuracy diagnostics urogenital chlamydia for both primary diagnosis and control cured.

erlichioses — group zoonotic, predominantly transmissive, distributed ticks infections, leaking in form acutely feverish diseases. With myalgia, rash, swollen lymph nodes, liver and spleen, severe pancytopenia and sometimes With development multiple organ insufficiency.

erlichii were for a long time known veterinarians. How pathogens hemolytic anemia cattle in Asia under the names *Anaplasia marginatus* (1910) and *Cowdria rumminantium* (1925). AT 1935 G. F. Donatien and Lestoquard in Algiers. With help coloring on Giemsa discovered in circulating monocytes sick dogs intracellular vacuole-forming microorganism, which was assigned cricketsiam again educated in 1937 G. kind Ehrlichia, named So in honour great German microbiologist Paul Ehrlich.

In 1953, in Japan, a similar microorganism was isolated by M. Kobayashi from blood, bone brain and monocytes 25 year old man suffering mononucleosis-like disease, famous in countries Far East. How sennetsu fever". detailed description this diseases, pathogen whom in 1984 was assigned to kind Ehrlichia, was presented

N. Tachibana in 1986 G. in Malaysia. AT subsequent years in various regions peace, predominantly in America, new species of erlichia were identified and variants described infections at person.

Pathogens belong to the genus Ehrlichia, tribe Ehrlichieae, family Rickettsiaceae, order rickettsiales, are small (1-3 µm) kokmavidnye microorganisms which parasitize in leukocytes (monocytes, granulocytes, lymphocytes). With education colonies enclosed in vacuoles (up to 30-40 ehrlichia in one vacuole), known as morula (from LC. morula — plural number titles blackberries).

Currently, more than 10 species of Ehrlichia pathogenic for animals and humans have been described. which, according to the results of the analysis of 16rRNA-rem, are divided into 3 groups: Ehrlichia sennetsu group (*E. sennetsu*, *E. risticii*, *Neorickettsia helminthoecae*), Ehrlichia group canis (*E. canis*, *E. chaffeensis*, *E. ewingii*) and the Ehrlichia phagocytophila group (*E. phagocytophila*, *E. equi*, HGE-agent — agent that causes human granulocytotropic ehrlichiosis, *Anaplasma marginatus*), a same ungrouped kinds.

Most ehrlichiosis are zoonotic bloodborne vector-borne infections, pathogens which are transmitted by bloodsucking ticks (nymphs, adults). The latter have observed transstadial broadcast erlichius,

but transovarial broadcast pathogens not installed.

erlichiosis sennetsu develops after ingestion pathogens With thermally bad processed nautical fish. For human pathogenic are erlich groups *E. canis* (pathogens monocytotropic erlichiosis human, or human monocytotropic ehrlichiosis — NME), group *E. phagocytophila* (causative agents of human granulocytotropic ehrlichiosis, or Human granulocytotropic ehrlichiosis — HGE), and *E. sennetsu*, pathogen **fever sennetsu** (Sennetsu fever). Due to the fact that Ehrlichia of one species can parasitize in cells various type, terminology, founded on cytotropism pathogens not always is correct.

Clinical diagnosis is based on the identification of an acute febrile illness with severe leukopenia and thrombocytopenia at patient, exposed attacked ticks. It is necessary to differentiate MEC from PLSH, ARVI, sepsis, leptospirosis, disease Lyme, coxiellosis.

Verification diagnosis achieved highlighting culture erlichius, but this is requires special Wednesdays and conditions, so in wide practice used seldom. At microscopy painted on Giemsa drugs blood morula erlich in monocytes are found seldom (about 7% cases).

main method specific but usually retrospective diagnostics is NRIF, which detects the increasing titer of antibodies to *E. chaffeensis* or *E. canis* classes IgM and IgG (from > 1:80 at the end of the 2nd week to 1:1280 at the 4-6th week of illness). Developed methods diagnostics MEC With using PCR.

Etiotropic drugs choice are tetracycline derivatives: tetracycline (Hexacycline) 0.25 g 4 times a day or doxycycline (Vibramycine) 0.1 g twice in day in flow 5-7 days. Pregnant and children younger 8 years old instead of tetracyclines, rifampicin (Rifadine) 300 mg twice a day within 5-7 days. Unlike rickettsiosis, levomycetin is contraindicated in ehrlichiosis (Chloramphenicol). At heavy flow disease carry out pathogenetic and sometimes intensive therapy.

Prevention directed on warning attacks ticks With help repellents and use protective clothes. Developed vaccination.

Granulocytic erlichiosis

Pathogens - erlich groups *E. phagocytophila* (*E. phagocytophila*, *E. equi*, HGE-agent).

Reservoir *E. phagocytophila* serve white-footed mice, deer, bison, dogs, horses, sheep, cattle. The mechanism of infection is blood transmissible, It is realized when blood-sucking ixodid ticks. The carriers of pathogens are ixodic ticks *Ixodes scapularis*, *I. pacificus* in American region, *I. ricinus* in Europe.

Diseases are registered all year round predominantly among rural residents, especially men mature age (80% cases), With seasonal rise incidence during the period of activity of ticks (July-November). Most of the cases of HH (about 500) registered in the USA (11 states), sporadic diseases are observed in countries Northern and Central Europe.

From the place of inoculation of Ehrlichia, where the primary affect is often formed, pathogens lymphogenously penetrate into the blood and hematogenously disseminate, penetrating into myeloid cells bone brain, circulating neutrophilic leukocytes. Developing erythrophagy, formed granulomas and foci necrosis in liver, focal necrosis in spleen, inflammatory infiltrates and hemorrhages in the lungs. As a result of defeat neutrophils observed activation opportunistic infections.

At convalescents develops persistent immunity.

Manifestation infections observed at small parts seropositive persons and usually develops in benign diseases.

Incubation period is 4-8 days.

Start disease acute, "flu-like" characterized fast rise temperature body before 39-40 °C, widespread myalgia, arthralgia, head pain, weakness. At the site of tick suction, a primary affect in the form of papulo-vesicles or ulcers under the crust.

At 1/3 sick celebrated nausea, vomit and diarrhea, in 2-10% cases appears maculopapular widespread rash, sometimes in the form of erythematous fields. More than 1/4 sick observed shortness of breath and unproductive cough.

AT most cases through 1-2 weeks comes recovery.

At row sick observed coinfection *Borrelia burgdorferi* and *Babesia microti*.

Laboratory research reveal expressed leukopenia (granulocytopenia) thrombocytopenia, increased activity of ALT and AST. Sometimes there is pancytopenia at saved or elevated cellular composition of the bone brain.

In immunocompromised patients, the disease may be complicated by the development respiratory distress syndrome, ITSH, sometimes — meningoencephalitis With loss consciousness. Forecast usually favorable, mortality less 1%, observed at patients With dysfunctional premorbid background.

Clinical diagnosis installed on basis epidemiological information about attack ixodid ticks, after whom arises flu-like disease With severe neutropenia, thrombocytopenia and increased activity of aminotransferases. Differential diagnosis is carried out with Lyme disease, SARS, enterocolitis, pneumonia coxiellosis

Specific diagnosis is achieved by culture isolation *E. phagocytophila* (in wide rarely used in practice) or detection of *E. phagocytophila* morula in neutrophils peripheral blood In contrast to MECs, morulae in blood granulocytes are found in 50-75% sick GECh.

Developed methods diagnostics With help PCR.

Serological diagnostics (NRIF in a titer of 1:80 or more) has a retrospective value, IgM class antibodies to *E. phagocytophila* remain in the blood up to 1.5 months from the onset illness. In parallel, a serological examination is carried out to detect co-infection. *Borrelia* and *Babesia*

Etiotropic therapy, carried out as in MEC, provides a reduction in symptoms disease through 24-48 hour.

Prevention. Protection from attacks ticks.

TEST TASKS

1. Which material for microbiological research should take at patients at suspicion on chlamydia?
 1. Detachable urethra
 2. Vaginal smear
 3. smear from pharynx
 4. Rectal smear
2. What kind of properties characteristic for chlamydia?
 1. Gram negative
 2. prokaryotes
 3. Obligate intracellular parasites
 4. Have twisted shape.

PRACTICAL LESSON №10

DELIVERY OF THE MODULE ON TOPIC: INTESTINAL INFECTIOUS PATHOGENIC anaerobes, zoonoses, rickettsiosis, chlamydia, mycoplasmosis, ERLICHIOZ.

PRACTICAL OCCUPATION #11

THEME: LABORATORY DIAGNOSTICS diphtheria And whooping cough

Educational goal:

1. Teach students the methods of microbiological diagnostics and specific prevention diphtheria, whooping cough

Student should know:

1. Biological properties and laboratory diagnostics diphtheria, whooping cough
2. specific prevention diphtheria, whooping cough

The student must be able to:

1. cook smear and paint according to the method Neisser.
2. cook smear and paint over method Gram.
3. Define toxigenicity diphtheria cultures on Ouchterlony.
4. Put a test for cystinase and a test for urease of diphtheria and false - diphtheria sticks.

PLAN:

1. Taxonomy and main biological properties pathogens diphtheria, whooping cough
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles microbiological diagnostics diphtheria, whooping cough
4. Drugs foretropic therapy and specific prevention diphtheria, whooping cough

INDEPENDENT JOB

1. Cooking smear and coloring on method Neisser.

2. Cooking smear and coloring on method Gram.
3. Definition toxigenicity diphtheria cultures on Ouchterlony.
4. Carrying out tests for cystinase and urease of diphtheria and false - diphtheria sticks.

METHODOLOGICAL RECOMMENDATIONS

diphtheria wand (*Corynebacterium diphtheriae*) — gram-positive rod-shaped bacteria kind *Corynebacterium*. First pathogen was discovered on sections of films obtained from the oropharynx of patients in 1883 by Edwin Klebs (German: Edwin Klebs, 1834-1913). A year later, Friedrich Löffler (German: Friedrich August Johannes Löffler, 1852-1915) a pure culture was isolated. Diphtheria toxin was obtained by E. Ru and A. Yersin (1884-1888 gg.). Anatoxin discovered Ramon Gaston in 1923 G. and offered use it for active immunization. *Corynebacterium diphtheriae* - large (1-8 × 0.3-0.8 microns) straight, slightly curved polymorphic rod-shaped bacteria. At the poles cells, metachromatic grains of volutin are localized, giving the cells a characteristic form "maces". grains volutin stained methylene blue on Neisser. On micropreparations are located alone or due to features division cells are located in form of latin letters V or Y. Dispute and capsules do not form.

Epidemiology. The source of infection in diphtheria are people - sick or healthy carriers toxigenic diphtheria microbes. the greatest epidemic danger present sick diphtheria pharynx, nose and larynx, actively highlighting pathogens into the environment with exhaled air. Minor in this relation meaning play sick diphtheria eye, skin, wounds and others localizations capable of spreading the infection by contact (through hands, objects life).

Pathogenesis. Entrance gates of pathogens of diphtheria can be almost everything areas of integument (skin and mucous membranes) of the macroorganism. However, most often they are mucous shell oropharynx, much less often - larynx, nose, conjunctiva genital bodies, wound surface, skin and others Toxigenic corynebacteria fixed on tissue cells, multiply and in the process of life produce exotoxin, having a local and general effect, causing almost all manifestations pathological process. microbial cells beyond the limits fabrics, being the gateway infection, usually, does not apply to direct participation in defeat macroorganism not accept.

Diphtheria exotoxin includes from several factions, each from which has independent biological action. One from them - hyaluronidase: destroys hyaluronic acid capillary and raises them permeability. it leads to exit per limits vessels liquid parts blood, impregnation affected fabrics plasma containing fibrinogen along with other components. The second is necrotoxin - causes necrosis of the epithelium at the site of the gate infection, accompanied by a release from epithelial cell thrombokinase. The latter promotes the conversion of fibrinogen into fibrin and education on surfaces affected fabrics fibrinous films. Palatal tonsils, unlike other organs, are covered with multi-row epithelium. As a result the fibrin film formed during diphtheria penetrates deep into the epithelial cover and tight soldered to tissues. Third faction diphtheria toxin - true diphtheria toxin (its main component) is able to displace from cellular structures cytochrome B and thus block the processes of cellular respiration and synthesis in them protein molecules. Most sensitive to this change are myocardium, capillaries and nervous cells. AT cardiomyocytes develop phenomena myocardial dystrophy with their subsequent necrosis, myolysis and the development of infectious-toxic myocarditis. Defeat capillaries at diphtheria accompanied infectious-toxic shock. Damage nervous cells accompanied dystrophic changes Shvanovsky cells and demyelination nervous fibers. Along With marked general action diphtheria toxin appears phenomena general intoxication.

Basis laboratory diagnostics constitute bacteriological research: selection pathogen from hearth inflammation, definition his type and toxigenicity. Material take away sterile wadded tampons dry or wetted (before sterilization!) five% solution glycerin. At storage and transportation tampons protect from cooling and drying. The material should be sown no later than 2-4 hours after taking. In patients with angina who were in contact with patients with diphtheria, as well as in persons with typical clinical manifestations of diphtheria, the diagnosis is made even with a negative bacteriological research.

Auxiliary meaning It has definition credits antitoxic antibodies in paired sera at staging RGA. toxin formation reveal using RGA With antibody erythrocyte diagnosticum. For identifying diphtheria toxin proposed use PCR.

Main in treatment diphtheria think introduction antitoxic diphtheria serum. She neutralizes toxin, circulating in blood, Consequently, renders largest Effect at early application

Preventive Events. Vaccination remains main way control diphtheria. Immunization schedule children provides immunization DTP vaccine starting from 3 months of life (vaccinated 3 times with an interval of 30-40 days). Revaccination is carried out 9-12 months after the completed vaccination. For revaccination in 6-7, 11-12 and 16-17 years old apply ADS-M. AT individual cases, for example at contraindications to whooping cough component DPT, ADS-M apply and for vaccination.

Whooping cough (whooping-cough - English; Keuchhusten - it; Coqueluche - French) and paraptussis - sharp infectious disease, clinically indistinguishable from pertussis. Characterized sharp respiratory ways and attacks spasmodic cough.

The causative agent of whooping cough (*Bordetella pertussis*) is a short stick with rounded ends (0.2-1.2 μm), gram negative motionless, Good stained with aniline dyes. Antigenically heterogeneous. Antigen, which causes the formation of agglutinins (agglutinin), consists of several components. They are named factors and are designated figures from 1 before fourteen. Factor 7 is generic, factor 1 contains *C. pertussis*, fourteen - *C. paraptussis*, rest found in different combinations; for the causative agent of whooping cough, these are factors 2, 3, 4, 5, 6, for paraptussis - 8, nine, 10. Reaction agglutination With adsorbed factorial sera allows differentiate kinds *Bordetella* and determine them antigenic options. pathogens whooping cough and paraptussis very unstable in external environment, so seeding is necessary do immediately after taking the material. bacteria die quickly when dried, ultraviolet irradiation, under the influence of disinfectants. sensitive to erythromycin, chloramphenicol, antibiotics tetracycline groups, streptomycin.

Pathogenesis. Gates infections is mucous shell respiratory tract. whooping cough microbes attached to cells flickering epithelium, where they multiply on surfaces mucous shells, not penetrating in blood flow. On place implementation pathogen develops inflammatory process, oppressed activity ciliary apparatus cells epithelium and increases secretion mucus. AT further there is ulceration of the epithelium of the respiratory tract and focal necrosis. Pathological process most pronounced in bronchi and bronchioles, less pronounced changes develop in trachea, larynx and nasopharynx. Mucopurulent cork cork up clearance small bronchi, develops focal atelectasis, emphysema. Observed peribronchial infiltration. AT genesis convulsive seizures It has meaning sensitization organism to toxins pertussis sticks. Permanent irritation receptors respiratory ways conditions cough and leads to formation in respiratory center hearth arousal type dominants. Due to this typical seizures spasmodic cough may to be caused and non-specific irritants. From the dominant focus, excitation can radiate to other departments of the nervous system, for example, vasomotor (increased blood pressure, vasospasm). irradiation arousal explained same appearance spasmodic muscle contractions faces and torso, vomiting and others symptoms whooping cough Postponed whooping cough (How and pertussis vaccinations) does not provide lifelong immunity, so possible repeated diseases whooping cough (about five% cases whooping cough account for on adults people).

Reliable diagnosis in catarrhal period maybe to be staged after receiving results bacteriological research. foundation for research in these cases usually serve epidemiological data (contact With sick whooping cough lack of data on vaccinations, etc.). In the period of spasmodic cough, the diagnosis of whooping cough put much easier, So How appear typical attacks. However need to take into account that sometimes coughing fits similar to whooping cough may be due to others reasons (adenoviral infection, viral pneumonia, compression respiratory tract in malignant neoplasms, infectious mononucleosis and etc.), on the other hand, whooping cough can occur atypically without characteristic seizures (in vaccinated children, in adults). The main method of laboratory confirmation of the diagnosis is selection pathogen whooping cough Frequency allocation depends from timing taking material; in the 1st week of the disease, positive results can

be obtained in 95% patients, on the 4th - only 50%, and starting from the 5th week, the microbe can no longer be isolated. Material from nasopharynx take dry swab With immediate sowing on cups With selective nutritional environment. use same method "cough records", at which cup petri With nutritional environment installed front mouth coughing child (at a distance of about 10 cm), held in this position for several seconds, to catch 5-6 cough shocks. Cup With sowing quickly close lid and put into the thermostat. At transportation protect from cooling (wrapped in paper, cotton wool, a heating pad filled with hot water is placed in the container). However, in terms of frequency allocation pathogens whooping cough method "cough records" much inferior taking material swab. Serological methods can use for retrospective diagnostics, a same at sick With negative results bacteriological research. From old methods can use RSK, RPGA, agglutination reaction. A 4-fold increase in antibody titers is considered diagnostic. more, a same high titers antibodies (1:80 and higher).

Recently, enzyme immunoassay has been successfully used to detect antibodies in serum (immunoglobulins class M) and in nasopharyngeal slime (immunoglobulins class A). These antibodies appear co 2nd-3rd weeks disease and persist in flow 3 months

Test control

1. Methods used for staining diphtheria bacillus:
 - A) Gram's method
 - B) Neisser method
 - C) method Ozheshko
 - D Method Tsilya –Nelsen
2. Biological options diphtheria sticks:
 - A) Gravis
 - B) Mitis
 - C) Intermedius
3. What kind associated drugs use for prevention diphtheria, whooping cough
 - A) DTP
 - B) typhoid vaccine with tetraanatoxin.

A task #1

When examining for diphtheria carriage from the pharynx of a kindergarten teacher identified a microbe with the following properties: volutin grains are found in individual individuals, sucrose, glucose, starch does not break down, tests for cystinase and urease are negative.

PRACTICAL OCCUPATION #12

THEME: LABORATORY DIAGNOSTICS OF TUBERCULOSIS And LEPRO

Educational goal:

1. Teach students the methods of microbiological diagnostics and specific prevention tuberculosis.

Student should know:

1. Biological properties and laboratory diagnostics tuberculosis,
2. specific prevention tuberculosis.

The student must be able to:

1. cook smear and paint on method Tsilya-Nelsen.
2. cook smear and paint over method Gram.

PLAN:

1. Taxonomy and main biological properties pathogens tuberculosis.
2. Epidemiology, pathogenesis, immunity caused diseases.
3. Principles microbiological diagnostics tuberculosis.

4. Preparations for etiotropic therapy and specific prevention tuberculosis.

INDEPENDENT JOB

1. Microscoping micropreparations: mycobacteria tuberculosis, meningococci.
2. Explore scheme laboratory diagnostics tuberculosis.
3. Will study method microculturing for express diagnostics tuberculosis;
4. Microscoping and sketch demonstration a drug "microculture myc. Tuberculosis."

METHODOLOGICAL RECOMMENDATIONS

Mycobacteria belong to the Mycobacteriaceae family, Mycobacterium genus. Distinguish:

- 1) Mycobacteria human type (Mycobacterium tuberculosis), 2) mycobacteria bullish type (Mycobacterium bovis), 3) mycobacteria avian type (Mycobacterium avium), 4) atypical mycobacteria, which are divided into four groups (according to Runyon):
 - a) photochromogenic bacteria, which are growing in darkness in flow 21-46 days in form non-pigmented colonies, but after lighting daytime or electric light acquire yellow or orange coloration. pathogenic for people;
 - b) scotochromogenic bacteria, which grow slowly (60-100 days), form yellow- Orange pigment in darkness. Some from them pathogenic: cause defeat lymphatic nodes and lungs at children;
 - c) non-photochromogenic Mycobacteria do not form pigment either in the light or in the dark. pathogenic for person;
 - d) fast growing mycobacteria, are growing in flow several days in form non-pigmented colonies. To this group relate How potentially pathogenic mycobacteria, So and saprophytes.

Pathogen tuberculosis: Mycobacterium tuberculosis is yourself thin, slightly curved sticks long 2.5-3.5 micron, different big polymorphism: long, branched and granular forms. Mycobacterium bovis - short, thick sticks, Mycobacterium avium - filiform, branched forms.

For productions microbiological diagnosis use microscopic, bacteriological, biological, serological and allergic research methods. A wide variety of material can be received for research, depending on whether where the pathological process is located: with pulmonary tuberculosis - sputum, with tuberculosis kidneys - urine, at tuberculosis meningitis - spinal liquid

I. MICROSCOPIC METHOD

researched material: sputum, urine, spinal liquid. For "enrichment" sputum wide are used methods homogenization and flotation. At staining of smears according to Ziehl-Neelsen, tubercle bacilli are stained bright red Colour. The use of fluorescent microscopy increases the number of tubercular sticks.

Microscopic examination is indicative and allows us to judge only about the presence of acid-resistant bacteria in the material without determining their species and type accessories.

II. BACTERIOLOGICAL STUDY

Peculiarities method

For release from accompanying microflora researched material handle 10% sulfuric acid or 4-6% solution caustic sodium, a then centrifuged. acid neutralized and the material is poured into several tubes with environment Lowenstein-Jensen or others special Wednesdays.

Crops are incubated at 37 °C 4-6 weeks or more, since tubercle bacillus breeds very slowly, especially in first generations. Colonies have view greyish or light cream wrinkled or crumbly dry raid.

At identification more often Total define ability dedicated culture synthesize nicotine acid - niacin try Konno - With help which it is possible to distinguish M.tuberculosis, which synthesize nicotinic acid well, from rods M.bovis, forming it in minimal quantities. Atypical mycobacteria have high catalase activity. Peroxidase activity at them not is revealed. Determination of the thermal stability of catalase makes it possible to differentiate virulent for human mycobacteria (human and bovine types), in which it is thermolabile, from acid-resistant saprophytes and atypical mycobacteria, which form thermostable catalase.

For accelerated diagnosis of tuberculosis, the Price microculture method is used. For To do

this, thick smears of the material under study are made on several glass slides. smears handle 2-6 % sulfuric acid and neutralize alkali. After this them placed in vials with hemolyzed citrated blood. After 7-14 days material stain on Ziel-Nielsen and microscopic - virulent strains form microcultures, cord-like or braid (Availability cord factor).

III. BIOLOGICAL METHOD

Applies With goal allocation clean culture pathogen tuberculosis from bodies animal, infected researched material, a same for definitions virulence of mycobacteria. The test material is treated with sulfuric acid to release from outsider microflora, neutralize and introduce subcutaneously nautical mumps and rabbit with negative tuberculin reactions. Through 4 months, if the animal will not die, it is slaughtered, macro- and microscopic examination is carried out bodies and do crops. M.tuberculosis highly pathogenic for maritime pigs and few pathogenic for rabbits. M.bovis highly pathogenic for rabbits.

IV. SERODIAGNOSIS

use in quality additional text RSK and RPGA. Positive results are celebrated at active tuberculosis, a same at infection mycobacteria tuberculosis and vaccination.

V. SKIN-ALLERGIC TRY

Put with tuberculin (RPO) - purified protein fraction obtained from mycobacteria tuberculosis, for characteristics, estimates currents tuberculosis process, definitions efficiency vaccination and selection contingents for revaccination vstuberculosis. Tuberculin introduce intradermally in strictly certain dosage (reaction Mantu). The result is taken into account after 24-48 hours by the formation of hyperemia and papules

SPECIFIC PREVENTION

Vaccine BCG. live, freeze-dried dried culture apathogenic strain mycobacteria tuberculosis, received French scientists A) Calmette and M. Guerin. Applies intradermally for active specific prevention tuberculosis.

test control

1. Morphological saints pathogens tuberculosis
 - a) cocci
 - b) Thin long sticks
 - c) short sticks
 - d) Granular forms
 - e) The presence of spores
2. Chemical in-va, determining acid resistance mycobacteria
 - a) Lipids
 - b) Proteins
 - c) carbohydrates
3. Tests, characteristic for human kind pathogen
 - a) Niacin test (education nicotine acids)
 - b) Generalized process in a guinea pig
 - C) Generalized process rabbits.
4. Drugs, used for skin-allergic samples at tuberculosis.
 - a) Tulyarin
 - b) Brucellin
 - c) TuberculindDRD
5. AT what age produced vaccination vs tuberculosis
 - a) First a week life
 - b) 2-3 of the year
 - c) 6-7 years old

PRACTICE #13.

THEME: DIAGNOSTICS OF SYPHILIS

Educational goal:

1. Teach students the methods of microbiological diagnostics and specific prevention syphilis.

Student should know:

1. Biological properties and laboratory diagnostics syphilis.
2. Prevention syphilis.

The student must be able to:

1. Put reaction Wasserman complement binding.
2. Prepare a smear and stain according to the Romanovsky-Giemsa method.
3. Cook smear and paint on method Gram.

PLAN:

1. Taxonomy and main biological properties causative agents of syphilis.
2. Epidemiology, pathogenesis, immunity syphilis.
3. Principles microbiological syphilis diagnosis,
4. Preparations for etiotropic therapy and specific prevention syphilis.

INDEPENDENT JOB

1. staging reactions binding complement Wasserman.

METHODOLOGICAL RECOMMENDATIONS

Syphilis - chronic systemic venereal infectious disease With defeat skin, mucous membranes, internal organs, bones, nervous systems With successive stages of disease caused by bacteria species *Treponema pallidum* (pale treponema) subspecies *pallidum* , related to kind treponema (*Treponema*) families *Spirochaetaceae* .

Etiology. Syphilis is transmitted mainly through sexual contact, and therefore refers to a group of sexually transmitted diseases , or STIs (sexually transmitted infections). However possible broadcast syphilis and through blood , for example, at transfusion blood syphilis-infected donor , or injection drug addicts when using common syringes and / or common containers for drug solutions , or in everyday life when using general "bloody" tool type dental brushes or razors.

The household "bloodless" route of infection with syphilis is also not excluded, but it is very rare and requires close contact With sick tertiary syphilis having open syphilitic ulcers or decaying syphilitic gummas , of which the causative agent can get, for example, on the dishes from which the patient drank. You can also list towels, spoons, toothbrushes, linen, etc. in contact with mucous membranes items. The ability of the patient's urine and sweat to transmit infection has not been proven, in saliva pale treponema are found only at availability rashes in cavities mouth. It is possible that the infection of the child with mother's milk, even in the absence of visible changes in area of the breast, semen is also contagious, even in the absence of visible pathological foci on the patient's penis. Medical personnel may become infected disease at implementation medical and diagnostic events, a same at autopsy of the corpses of patients with syphilis, the corpses of children with primary congenital form diseases.

Pathogenesis. Incubation period primary stages syphilis is in average 3 weeks (interval from several days to 6 weeks) from the moment of infection. At the end incubation period in case of sexual or

domestic infection at the site of penetration microbe usually develops primary affect.

Pathogenesis syphilis conditioned reaction organism on implementation in organism patient with pale treponema. The features of the pathogen determine the polymorphism leaking in infected body processes, in dependencies from stages diseases pathological changes different enough much. AT classical flow syphilitic infections accepted allocate four period:

- Incubation;
- Primary;
- Secondary;
- Tertiary.

Latest three period are found characteristic symptoms incubation period does not manifest itself in any way, and its terms are determined only indirectly after the appearance clinics.

Diagnostics. Diagnosis syphilis in some cases can suspect clinically, but main method diagnostics and confirmation preliminary diagnosis is serodiagnosis. Currently, to determine the antibodies to the pathogen is used ELISA, earlier in Russia the Wasserman reaction was used for this. All diagnostic methods syphilis are separated on the following groups:

- Direct and indirect (indirect)
- Treponemal (specific) and non-treponemal (non-specific)
- Qualifiers (screeninD) and confirming (diagnostic)
- instrumentation, instrumentless.

Direct treponemal methods diagnostics allow discover pathogen directly in the biomaterial. These methods are dark-field microscopy, syphilis infection rabbits, cultural methods, PCR diagnostics.

Each of these methods has its own specific disadvantages that limit it. mass application. Dark field microscopy method can detect pathogen only at fresh syphilis and With his help impossible estimate dynamics and treatment effectiveness. Method of infection with syphilis in rabbits is expensive and slow, and also does not allow to assess the patient's condition in dynamics. cultivation pale treponema on artificial environments extremely difficult, in connections With sensitivity pathogen to conditions environment. Method PCR diagnostics allows effectively detect the pathogen only in primary and secondary syphilis, test systems are relatively expensive, and studies of the effectiveness of this method in diagnosing syphilis is still going on. Thus, we see that direct diagnostic methods hardly applicable in clinical practice, and therefore, the basis of diagnosis are various serological methods (indirect).

In accordance with the current order of the Ministry of Health of the Russian Federation No. 87 dated March 26, 2001 "On improving serological diagnostics syphilis" at gray and liquor diagnostics syphilis allowed usage next reactions.

- Microreactions precipitation (indirect screening method)
- Reactions passive indirect agglutination (RPGA)
- Reactions immunofluorescence (RIF)
- Reactions immobilization pale treponem (RIBT)
- ELISA analysis not require separate regulation in connections With how, in Order No. 87 not indicated.

It should be noted that none of the diagnostic methods guarantees 100% detection. pathogen. Sensitivity methods is 90-98%, so simultaneous usage 2 various methods research maybe With very high degree credibility install loyal diagnosis.

TEST TASKS

1. Which material for microbiological research should take at patients at suspicion nasypylis?
 - 1 Urethral discharge
 - 2 Vaginal swab
 - 3 Throat swab
 - 4 Detachable chancre
2. What kind of properties characteristic for spirochetes?
 1. Gram negative

2. prokaryotes
3. Obligate intracellular parasites
4. Have twisted form.
3. Ways microscopy spirochete:
 1. By Ramonovsky – Giemse
 2. By Gramu
 3. Phase contrast microscopy
 4. Dark field microscopy.
5. Fibrils located near spirochete:
 1. On the surface of the outer cell wall
 2. Under outdoor shell
 3. Between shell and protoplasmic cylinder
6. Antigens used for productions RSK at diagnostics syphilis:
7. A. O antigen
- B. Cardiolipin
- C. Soluble antigen
- D. Treponemal specific

PRACTICAL OCCUPATION #14

DELIVERY OF THE MODULE ON THE TOPIC: DIAGNOSTICS OF DIPHTHERIA, PERTUSSIS, TB, leprosy, SYPHILIS.

PRACTICE #15. THEME: SARS

DIAGNOSIS And FLU

Educational goal:

1. Train students in the methods of virological diagnostics and specific prevention flu, SARS.

Student should know:

1. Biological properties and laboratory diagnostics flu, SARS.
2. Specific prevention flu, SARS.

The student must be able to:

1. Put and take into account results REEF at SARS.
2. Put and take into account results RTGA for seroidentification at flu.
3. Put and take into account results ELISA for serodiagnosis at SARS.

PLAN:

1. Taxonomy and main biological properties pathogens flu, SARS,
2. Epidemiology, pathogenesis, immunity called diseases.
 1. Principles microbiological diagnostics flu, SARS
 2. Preparations for etiotropic therapy and specific prevention flu, SARS, measles, rubella, wind smallpox, epidemic mumps.

INDEPENDENT JOB

1. Parsing supplies and accounting results REEF with SARS (demonstration).
2. Parsing supplies and accounting results RTGA for seroidentification at flu (demonstration).
3. Parsing supplies and accounting results ELISA for serodiagnosis at

SARS (demonstration).

METHODOLOGICAL RECOMMENDATIONS

Flu (from fr. grippe) — acute infectious disease respiratory ways, caused by the influenza virus. Included in the group of acute respiratory viral infections (ARVI). Periodically spreads in the form of epidemics and pandemics. Currently more than 2000 variants of the influenza virus have been identified, differing in antigenic spectrum.

The virus was first isolated in the 1930s. Influenza viruses belong to the family Orthomyxoviridae, which includes the genera Influenza A, B, C. Antigenic properties of internal proteins virion (M1 and NP) define belonging flu virus to kind AND, AT or FROM.

epidemic meaning for people have viruses, containing three subtype HA(H1, H2, H3) and two NA subtypes (N1, N2). Influenza A and B viruses contain NA and HA as major structural and antigenic components viral particle, possessing hemagglutinating and neuraminidase activities. At virus influenza FROM No neuraminidase, he has instead of this hemagglutinin-esterase (penetratinD) protein (HEF). A thread RNA surrounded protein and packed in lipoprotein membrane. Virions able agglutinate erythrocytes and elute in them With help virus-specific enzymes.

The influenza virus has a spherical shape with a diameter of 80-120 nm, in the center are RNA fragments, prisoners in lipoprotein shell, on surfaces which there are "spikes" consisting of hemagglutinin (H) and from neuraminidase (N). Antibodies, produced in answer on hemagglutinin (H) constitute basis immunity vs certain subtype pathogen influenza

source infections is sick human With explicit or erased form disease that releases the virus with coughing, sneezing, etc. The patient is contagious from the first hours diseases and before 5th–7th days disease.[5] Characterized aerosol (inhalation tiny drops of saliva, mucus that contain the influenza virus) transmission mechanism and extremely rapid spread in the form of epidemics and pandemics. influenza epidemics, caused serotype AND, arise approximately every 2-3 of the year, a caused serotype AT

- every 4-6 years. Serotype C does not cause epidemics, only sporadic outbreaks in children and weakened people. AT form epidemics meets more often in autumn-winter period. The periodicity of epidemics is associated with frequent changes in the antigenic structure of the virus during stay his in natural conditions.

Input gate for virus influenza are cells flickering epithelium upper respiratory tract - nose, trachea, bronchi. The virus replicates in these cells leads to their destruction and death. This explains the irritation of the upper respiratory ways of coughing, sneezing, nasal congestion. Penetrating into the blood and causing viremia, the virus renders direct, toxic action, emerging in form raise temperature, chills myalgia, headache. Besides, virus increases vascularity permeability, causes development stasis and plasma hemorrhage.

Vaccination is the traditional way to prevent influenza. Proposed vaccine for the prevention of influenza in the form of live, killed (inactivated), subunit vaccine. Vaccination is especially indicated in risk groups - children, the elderly people with chronic heart and lung diseases, as well as doctors. Usually carried out, when epidemiological forecast testifies about expediency mass events (usually in the middle of autumn). A second inoculation in the middle is also possible winters.

For rapid diagnosis of influenza use "express method" of virus detection influenza With help fluorescent antibodies. researched material take from nose in first days illness. cooked from him smears handle specific flu-like fluorescent sera. formed complex antigen- the antibody glows brightly in the nucleus and cytoplasm of cells of the cylindrical epithelium and clearly visible in luminescent microscope. Answer can receive through 2-3 h.

Serological research help retrospective diagnostics flu. Examine paired blood sera taken from patients in the acute period of the disease (up to the 5th day from the onset of the disease) and during the period of convalescence with an interval of 12-14 days. Most indicative in serological diagnosis are the complement fixation reaction (RSK) With flu-like antigens and reaction braking

hemagglutination (RTGA). Diagnostic counts titer increase antibodies in four times and more.

TEST TASKS

1. The avian influenza virus is
 - a) to the virus influenza type C
 - b) to the influenza virus type A
 - c) to the influenza virus type B
 - d) to virus influenza type D

2. Interferon provides antiviral protection of the cell, tk. prevents:
 - a) virus adsorption on cage;
 - b) penetration of the virus into the cell;
 - c) reproductions virus;
 - d) lysis affected cells; e) activation killers.

3. Install serological type virus influenza may with help:
 - a) reactions agglutination on glass;
 - b) reactions braking hemagglutination;
 - c) reactions indirect hemagglutination;
 - d) reactions hemagglutination.

4. In the pathogenesis of viral diseases, a decisive role is played by:
 - a) virulence virus;
 - b) toxigenicity virus;
 - c) level lysozyme;
 - d) reaction organism on cells, affected virus.

PRACTICAL OCCUPATION #16

THEME: DIAGNOSTICS PARENTERAL HEPATITIS, HERPES And HIV INFECTIONS

Educational goal:

1. Train students in the methods of virological diagnostics and specific prevention hepatitis B, C, D, G, HIV infections.

Student should know:

1. Biological properties and laboratory diagnostics hepatitis B, C, D, G, HIV infections.
2. Specific prevention of hepatitis B, C, D, G, HIV infection.
3. Biological properties and laboratory diagnostics herpes

The student must be able to:

1. Put and take into account results reactions ELISA for serodiagnosis and seroidentification with hepatitis B, C, D, G, HIV infections.
2. Put and take into account results RPGA at hepatitis C.
3. Put and take into account RTGA results and ELISA for serodiagnosis at Herpes.

PLAN:

1. Taxonomy and main biological properties pathogens hepatitis B, C, D, G, HIV infection, herpes
Epidemiology, pathogenesis, immunity called diseases.
2. Principles microbiological diagnostics hepatitis B, C, D, G, HIV infection, herpes.
3. Preparations for etiotropic therapy and specific prevention hepatitis B, C, D, G, HIV-infections, herpes.
4. Change module.

INDEPENDENT JOB

1. Analysis of the formulation and accounting of results ELISA reactions for serodiagnosis and seroidentification for hepatitis B, C, D, G, HIV infections (demonstration).
2. Analysis of the formulation and accounting of results RPHA reactions in hepatitis B (demonstration).
3. Parsing staging and accounting results RTGA and ELISA for serodiagnosis (demonstration).

METHODOLOGICAL RECOMMENDATIONS

Hepatitis B is a viral disease caused by the hepatitis B virus in the literature, it may be referred to as "HBV virus", HBV or HBV) from the family hepadnaviruses.

Virus is different extremely high sustainability to various physical and chemical factors: low and high temperatures (in volume including boiling), repeated freezing and thawing, prolonged exposure to an acidic environment. In external environment at room temperature virus hepatitis A AT may persist before several weeks: even in a dried and imperceptible stain of blood, on a razor blade, the end needles. AT serum blood at temperature +30°C virus infectivity preserved in flow 6 months at -20°C about 15 years old. Inactivated at autoclaving in flow thirty minutes sterilization dry heat at temperature 160°C in flow 60 minutes warming up at 60°C in flow 10 hours.

The mechanism of infection transmission is parenteral. Infection occurs naturally (sexual, vertical, domestic) and artificial (parenteral) ways. Virus present in blood and various biological liquids — saliva urine, semen, vaginal secret, menstrual blood and others contagiousness (infectiousness) virus hepatitis B exceeds contagiousness HIV in one hundred once.

Greatest meaning earlier everywhere had exactly parenteral way — infection at medical and diagnostic manipulation, accompanied violation integrity skin or mucous cover through medical, dental, manicure and other tools, transfusion blood and her drugs.

Pathogenesis. Most significant pathogenetic factor at viral hepatitis AT — death infected hepatocytes due to attacks own immune agents. massive death hepatocytes leads to violation functions liver, before Total detox, in lesser degree — synthetic.

Incubation period (time from infection to onset of symptoms) hepatitis AB amounts to average 12 weeks but maybe hesitate ranging from 2 before 6 months. The infectious process begins from the moment the virus enters the bloodstream. After hit viruses in liver through blood goes hidden phase breeding and accumulation viral particles. When a certain concentration of the virus in the liver is reached, acute hepatitis C. Sometimes spicy hepatitis passes for human practically imperceptibly, and is detected by chance, sometimes proceeds in a mild anicteric form - it manifests itself only malaise and decreased performance. Some researchers believe what asymptomatic flow, anicteric the form and "icteric" hepatitis constitute equal in the number of affected individuals in the group. That there are identified diagnosed cases acute hepatitis A AT constitute only one third all cases acute hepatitis A.

Vaccination. Mandatory vaccination. Recently, vaccination against hepatitis AT was enabled in mandatory the calendar vaccination. newborns most sensitive to virus hepatitis A AT - in case infections in this age, risk acquisition of a chronic form of hepatitis B is 100%. At the same time,

immunity created by the vaccine during this period of life, the most persistent. Recommended to vaccinate newborn more in maternity house, then through 1 month after first vaccinations, and through 6 months after the first vaccination (so-called 0-1-6 regimen). When you miss another injections should be remembered about the allowable intervals - 0-1(4)-6(4-18) months. However, if allowable intervals have been missed, it is necessary to continue vaccination according to the scheme, as if there were no passes. If vaccination was carried out according to the standard scheme, repeated vaccination is usually not required since immunity kept at least least in for 15 years. To determine how long immunity lasts during life, further research is needed - after all, vaccination began to be used relatively recently. Only after holding Total course vaccination, achieved almost 100%immunity. About 5% of the general population does not respond to vaccination, in these cases use other kinds vaccines vs hepatitis A C.

laboratory diagnostics GW - founded on identifying specific for GW antigens and relevant antibodies in blood, a same viral nucleic acids,main from which are:

- HB sAg - anti-HB s
- anti-HBc class Ig M and IgG
- HBe Ag - anti-HWe
- DNA HBV

The most widely used in the diagnosis of HB is the determination of HBsAg. This antigen is detected in both acute and chronic disease (however, acute infection usually confirmed presence high credits anti-HBs IgM). At acute GW the surface antigen of the virus is detected after 3-5 weeks from the moment of infection,that is, long before the appearance of clinical signs of the disease and in these cases is the only serological marker. HBsAg is constantly detected in preicteric and icteric periods illness. persistence HBsAg in flow 6 months and more indicates on protracted or chronic flow disease, and allows suppose chronic carriage of the virus. Elimination of HBsAg and the appearance of antibodies to it is indispensable condition convalescence. Serological markers replication HBV are - anti-HBc class IgM, HBeAg, DNA and DNA polymerase, which are detected at acute GW With first days clinical manifestations and may show up at exacerbation of chronic hepatitis B. Serological markers of HBV replication are defined as purposes general diagnostics, so for estimates efficiency applied therapy.

Hepatitis D virus (HDV) was first discovered in 1977. He does not belong to one of the known families of viruses. NDV is a spherical particle, in center which is spherical antigen (ND-AD), containing RNA. Outdoor the shell of the particle is formed by the surface antigen of the hepatitis B virus - HBs antigen (HBsAD). HDV cannot exist without the replication of the HB virus, which is why it is called virus - parasite or defective virus. Virus hepatitis A AT performs at this helper function, then eat role assistant for breeding NDV. Therefore, NDV - infection leaks always together With HBV- infection. NDV located in mostly innuclei hepatocytes and occasionally in cytoplasm.

Epidemiology. NDV- infection wide common. Intensity circulationNDV in different regions of the world varies significantly, but in general repeats the situation with HBV, although not completely accurate. In acute hepatitis, antibodies to HDV are secreted into different regions in 2-7% of patients, and in chronic hepatitis - in 9-50% of patients. On territories former the USSR among "healthy" carriers HBsAg greatest frequency (10-20 %) detection of antibodies to HDV detected in Moldova, Kazakhstan, Central Asia, Tuva, then found in areas hyperendemic for HBV. In the European part of Russia, the frequency of detection antibodies to NDV is 1.2-5.5 %.

The source of infection are patients with acute and chronic IOP, virus carriers, and also carriers of anti-HDV, since it is known that in individuals with anti-HDV, it is possible to simultaneously detect RNA-NDV. Transmission of HDV occurs in the same way as with HBV (parenteral, sexually, from mother to fetus). K delta - infections are susceptible to persons who have not been ill HBV (i.e. not having anti-HBs), as well as carriers of the HB virus (healthy carriers of HBsAg and patients with chronic HBV). Delta infection occurs both sporadically and outbreaks.

Pathogenesis, clinic. The infectious process caused by HDV manifests itself before Total appearance ND-Ag in blood. Delta - antihemia maybe to be short-term or lengthy depending on how

happened infection and whether integration of the HB virus into the hepatocyte genome. Distinguish between acute, protracted and chronic course of delta infection. The nature of its flow is limited by the duration of HBs- antigenemia: as it is depleted, it stops and synthesis of HDV, and the delta- dependent pathological process.

Delta infection develops as a coinfection or superinfection. With co-infection going on simultaneous infection HBV + NDV at persons, not ill previously HBV - infection (not having HBV infection markers before infection). In this case develops acute HBV + HD-hepatitis with the appearance of serological markers of two acute infections. At co-infections replication HBV more often Total HBV + IOP - hepatitis A usually it happens sharp and ends recovery.

At superinfection NDV - infection stratified on current HBV- infection at healthy carriers of HBsAg, in convalescents of the main HBV, in patients with chronic HBV. At the same time, a clinic of acute viral hepatitis delta develops, accompanied by appearance antibodies to delta antigen.

Laboratory diagnosis of hepatitis D (HD) The hepatitis D virus (HDV) is a defective a single-stranded RNA virus that needs help to replicate HBV for the synthesis of envelope proteins consisting of HBsAg, which is used for encapsulation genome IOP. IOP not belongs neither to alone from famous families animal viruses, in terms of its properties, IOP is closest to viroids and satellite viruses plants. laboratory diagnostics carried out through detection serological markers IOP, including Availability antigen, antibodies to him and HDR RNA. Detection antigen IOP and RNA IOP in serum blood or fabrics liver testifies about availability active HD infections, but, should Mark, what these markers may not show up in serum of patients with fulminant GD. Marker active replication IOP is also an anti-HDP IgM class. Serological markers HD infections depend on how the virus was acquired - in the form of co-infection with HBV (in most patients the disease has an acute course and ends with recovery) or superinfection at sick With chronic hepatitis B infection (flows heavier, how co-infection - 10% develop fulminant hepatitis). During superinfection in patients with chronic hepatitis B infection serological painting It has the following characteristic features: - the HBsAg titer decreases by the time the IOP antigen appears in the serum; - antigen IOP and RNA-HVD continue determined in serum So How usually at most patients with HD superinfection (70-80%) chronic infection develops, in contrast to cases of coinfection; - high titers of antibodies (anti-VOP) are determined as class IgM, So and IgG, which persist indefinite time. Serological markers of the HD virus are determined by enzyme immunoassay and radioimmunoassay, and RNA-HVD - method polymerase chain reactions.

Hepatitis C — anthroponotic viral disease With parenteral mechanism infection, most often flowing in form post-transfusion hepatitis A With predominance anicteric and prone to chonrization.

Hepatitis FROM called "affectionate killer" due to capabilities mask true reason under the guise sets others diseases.

Parenteral viral hepatitis C is caused by an RNA virus with a size virion 30-60 nm, belonging to the Flaviviridae family. HCV virus particles have membrane, contained in the blood in trace amounts and associated with lipoproteins low density and antibodies to proteins virus hepatitis A FROM. viruses, dedicated from complexes With lipoproteins and anti-HCV antibodies have diameter 60-70 nm. At electron microscopic studying on surfaces virion identified Good pronounced ledges tall 6-8 nm.

source infections are sick With active hepatitis C and latent sick — carriers virus. HCV infection is infection With parenteral mechanism infection - through infected blood and its components. Infection is possible with parenteral manipulations, including in medical institutions, including the provision of dental services, through injection equipment, acupuncture, piercing, drawing tattoos, when rendering range of services in hairdressing, but at genital contacts, the likelihood of getting hepatitis C is much less than hepatitis B, and boils down to minimal indicators.

laboratory diagnostics hepatitis C (GS). laboratory diagnostics HS was solved using modern methods of molecular biology, given that in HS the virus is in an extremely low concentration and its antigens are not available for detection with help contemporary methods indication, efforts researchers concentrated on detection of antibodies to various antigenic components of the virus,

the detection of which can serve as an indicator of the presence of the virus. Proteins were used as antigens. coded structural and non-structural zone HCV RNA, received at help recombinant technology or synthesis (polypeptides, used in contemporary immunological methods - C22-3; C33s, C100-3, C200, NS5, S-1-1). laboratory diagnostics HS based on discovery serological markv HCV: antibodies to virus HS (anti-HCV, anti-HCV class IgM, IgD) method ELISA and HCV RNA method PCR. To date, 4 generations of test systems have been developed for the detection of anti-HCV in enzyme immunoassay, but the first generation ELISA is not currently used due to low sensitivity. HCV RNA is indicator active replication HCV and most early marker infection, and maybe to be discovered method polymerase chain reactions already through 1- 2 weeks after infection, shortly before raise level serum transaminases. Anti-HCV are found to 5-6 week after start hepatitis A in 80% cases and to 12 week at 90% persons method enzyme immunoassay analysis. At in the determination of anti-HCV, in some cases a false positive reaction is recorded. To distinguish between false positives and true samples antibodies to HCV developed additional tests - recombinant immunoblotting, spectrum determination proteins anti-HCV.

HIV — virus immunodeficiency human, defiant disease — HIV infection last stage which known How syndrome acquired immunodeficiency (AIDS) — in difference from congenital immunodeficiency.

The spread of HIV infection is associated mainly with unprotected sex. contacts, use of virus-contaminated syringes, needles and other medical and paramedical instruments, transmission of the virus from an infected mother to her child during during childbirth or while breastfeeding. Mandatory testing in developed countries donated blood has greatly reduced the possibility of transmission of the virus during its use.

HIV primarily infects cells of the immune system (CD4+ T-lymphocytes, macrophages and dendritic cells), as well as some other types of cells. HIV-infected CD4+ T-lymphocytes gradually are dying.

The human immunodeficiency virus belongs to the family of retroviruses (Retroviridae), genus lentiviruses (Lentivirus). Name Lentivirus going on from Latin words lente — slow. Such title reflects one from features viruses this groups, a exactly — slow and unequal speed development infectious process in macroorganism. For lentiviruses same typical long incubation period.

Diagnostics. Flow HIV infections characterized lengthy absence significant symptoms disease[81]. Diagnosis HIV infections put on basis laboratory data: when detecting antibodies to HIV in the blood. Antibodies to HIV during the period the acute phase, as a rule, is not detected. In the first 3 months after infection with antibodies HIV is detected in 96-97% of patients, after 6 months. - the rest have 2-3%, and in later terms - only 0.5-1% (source Centers for Disease Control and Prevention USA, 2009). AT stages of AIDS register a significant decrease in the content of antibodies in the blood. First weeks post-infection represent the “seronegative window period” when antibodies to HIV are not detected. Therefore, a negative HIV test result in this period not means that man not infected HIV and can not infect others.

For the diagnosis of lesions of the oral mucosa in HIV-infected patients accepted working classification, approved in London, in September 1992 of the year. Everybody defeat divided on 3 groups:

1 group — defeat, clearly associated with HIV infection. This group includes the following nosological forms:

- candidiasis (erythematous, pseudomembranous, hyperplastic, atrophic); hairy leukoplakia;
- marginal gingivitis;
- ulcerative necrotic gingivitis;
- destructive periodontitis; sarcoma
- Kaposi;
- non-Hodgkin lymphoma.

2 group - lesions less clearly associated with HIV infection: bacterial infections;

diseases of the salivary glands;

viral infections; thrombocytopenic purpura.

3 group — defeats, which may to be with HIV infection, but not related With her.

Herpes (Greek *ἑρπης* - creeping, spreading skin disease) - viral disease with a characteristic eruption of grouped vesicles on the skin and mucous membranes shells.

Simple herpes (Herpes simplex) — group crowded bubbles With transparent contents on an inflamed base. Herpes is preceded by itching, burning of the skin, sometimes chills, malaise.

Shingles lichen (Herpes zoster) — characterized pain on move nerve, head pain. Through some days on site skin on move nerve appear rashes in the form of grouped vesicles, first with a transparent, and later purulent bloody content. Enlarged lymph nodes, fever body, the general condition is disturbed. Neuralgic pains can last up to several months.

Pathogenesis. The herpes virus is transmitted by direct contact, as well as through household items. It can also be transmitted by airborne droplets way. Herpes penetrates through the mucous membranes of the oral cavity, upper respiratory tract and genitals. Having overcome tissue barriers, the virus enters the blood and lymph. Then hits in various internal organs.

The virus penetrates the sensitive nerve endings and integrates into the genetic apparatus nervous cells. After this delete virus from organism impossible, he will remain With human on all life. immune system reacts on penetration herpes development specific antibodies, blocking circulating in blood viral particles. Characteristically awakening infections in cold time of the year, at colds diseases, at hypovitaminosis. reproduction herpes in cells epithelium skin and mucous shells leads to development of dystrophy and death cells.

According to research scientists Colombian University, herpes is stimulating factor for the development of Alzheimer's disease. Later, these data were independently validated by researchers at the University of Manchester. Previously the same a group of researchers led by Ruth Itzhaki proved that the herpes simplex virus found in the brains of nearly 70% of patients with Alzheimer's disease. In addition, they confirmed what at infection virus culture cells brain going on a significant increase in the level of beta-amyloid, from which plaques are formed. During recent study, scientists were able to find that 90% of the plaques in the brains of patients with sickness Alzheimer contain DNA simple herpes — HSV-1.

For diagnostics herpetic infections are used Everybody laboratory reactions — from cytological research before molecular biological methods.

Material for virus isolation for the purpose of diagnosis herpetic infections maybe serve content herpetic bubbles, scrapings With horny shells and fluids from the anterior chamber of the eye, blood, saliva, urine, cerebrospinal fluid feces pieces of tissue of the brain, liver, kidneys, spleen, lungs, lymph nodes taken for bio- or autopsy.

Infectious material can for a long time keep at -70°C , then How at temperature -20°C he quickly is inactivated. Virus containing fabrics may to be saved more 6 months at 4°C , if they are in 50% solution glycerin.

Exist whole row special methods for identifying viral antigens, specific antibodies and virus-induced morphologically modified cells.

Most accessible and technically uncomplicated is cytological method, allowing explore morphological changes in cells virus-infected simple herpes. The effectiveness of the method depends on obtaining sufficient quantities cells for research. Availability intranuclear inclusions, characteristic for reproductions virus herpes, serves confirmation diagnosis. Should remember, what intranuclear inclusion are found only after immediate commits smears scraping in absolute alcohol With subsequent coloration on Romanovsky-Giemsa. Morphological changes, induced virus simple herpes, can same discover in sections fabrics infected organs. characteristic for herpetic infections is: Availability multi-core cells, intranuclear inclusions and in some cases of hemorrhage. With a generalized form diseases multi-core cells With eosinophilic inclusions find in zones necrotic fabrics various bodies (brain, liver, kidney, adrenal glands, epithelium bronchi and trachea).

Method immunofluorescence — is method express diagnostics

herpetic infections and allows in flow 1-2 hours determine Availability herpesvirus antigens in the clinical material (scraping from the skin and mucous membranes, sections of biopsied organs). Identification of herpes simplex virus antigens to be completed in various modifications method immunofluorescence — straight, indirect, With application labeled complement.

From serological methods identification most often use reaction binding complement (RSK), especially in micromodifications her staging. micromethods use and for identifying virus simple herpes in reactions neutralization, passive hemagglutination and in others serological tests. Sensitivity listed methods are different.

AT the present time one from most sensitive methods diagnostics herpes infection is an enzyme immunoassay (ELISA) method that allows detect, depending on the type of biological material, how virus-specific antigens, so virus-specific antibodies class IgM, IgG.

TEST TASKS

1. HIV belongs to the group of viruses:
 - a) DNA-genomic;
 - b) RNA genomic;
 - đ complex.
2. The retrovirus family is distinguished by the presence
 - a) RNA polymerase
 - b) DNA polymerases
 - đ endonucleases
 - d) reverse transcriptase
 - e) exonucleases
3. Which type nucleic acids contains virus hepatitis A AT?
 - a) RNA
 - b) DNA
 - c) DNA and RNA
4. In the pathogenesis of AIDS, an important place is occupied by:
 - a) transformation PrP^c proteins in PrP^{sc} proteins;
 - b) unrestrained proliferation B-lymphocytes;
 - c) accumulation pathological myeloma proteins;
 - d) defeat T-helpers and macrophages.
5. In the pathogenesis of viral diseases, a decisive role is played by:
 - a) virulence virus;
 - b) toxigenicity virus;
 - c) level lysozyme;
 - d) reaction organism on cells, affected virus.

PRACTICAL LESSON #17

CHANGE MODULE ON TOPIC:

"VIRUS - ACTUATORS INFECTIOUS DISEASES HUMAN".

№ ЛД-21

**FEDERAL STATE BUDGETARY EDUCATIONAL INSTITUTION HIGHER EDUCATION
"NORTH OSSETIAN STATE MEDICAL ACADEMY»
MINISTRY OF HEALTH RUSSIAN FEDERATION**

DEPARTMENT MICROBIOLOGY

**COLLECTION METHODOLOGICAL DEVELOPMENT
ON MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY FOR
TEACHER FOR MEDICAL FACULTY**

SPRING SEMESTER

Vladikavkaz

Author: assistant professor, PhD Chertkoeva M.G.

Main appointment developments - methodical help teachers to each practical occupation in spring semester. Directions drawn up in accordance with the Federal State Educational Standard of the Higher and professional education.

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PRACTICAL OCCUPATION No. 1.

Theme: Morphology microbes. Microscopic method research.

Educational goal:

1. Explore morphology individual representatives bacteria.
2. master technology microscopy.
3. master simple method coloring microorganisms.

Plan lessons:

1. Familiarity with the rules of work and the basics of safety in microbiological laboratories.
2. Device and equipment microbiological laboratories, mode work and appointment.
3. Classification bacteria.
4. Morphology of bacteria, methods of study (light, dark-field, phase contrast, electronic microscopy).
5. Stages cooking smear.
6. Simple method coloring bacteria.
7. Cooking smears from culture staphylococcus and intestinal sticks, coloring simple method.
8. Demonstration drugs from micrococci, diplococci, tetracoccus, sarcin, staphylococcus, streptococci, intestinal sticks, bacillus, vibrios.

Independent Job students

1. Cooking smear and coloring simple method (under leadership teacher).
2. Development technology microscopy. microscopic studying morphology bacteria:
3. Viewing a demonstration smear from a pure culture of staphylococcus aureus (Staphylococcus aureus). Coloring gentian violet.
4. View demonstration smear from clean culture intestinal sticks (E. coli). Coloring — water fuchsin.
5. Decor protocol research.

EQUIPMENT

1. Set for bacteriological research: Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological
loop-8 pcs. Tray With stand - on
8 pcs
glass slides spirit
lamp -8 pcs
Bottle With physical solution -8 pcs.
2. Kit colors: Methylene blue -
8 pcs. Water magenta - 8 PC.
3. Tubes with the growth of cultures S. aureus -8
pcs. Tubes with crop growth E. coli - 8 pcs.

4. Microscopes - 8 pcs.
Immersion butter - four PC.
5. Demo micropreparations: sarcinas, streptococci, diplococci, intestinal wand, actinomycetes, staphylococci, spirochetes, vibrios, anthracoid.
6. Tables.

INFORMATIONAL MATERIAL ON THEME

1. **Technics cooking smears.** Smears cook on fat free subject glasses, previously outlining with a pencil on the glass, the place of the future smear on the opposite side of the subject glass. At growth bacteria on liquid nutritional environment, material take sterile bacterial loop, applied to glass and rubbed over the outlined area. When growth bacteria on dense nutritional environment, on subject glass previously inflict loop a drop of water and material rubbed.
2. The bacterial loop is sterilized in burner flame. The prepared smear is dried in air or held high above flame spirit lamps.
3. After this a drug fix, for what smear side, where No material, three times through the middle of the burner flame. Fixation allows you to kill germs, attach them to glass and, finally, killed microbes paint better, how alive.
4. Technics simple methods coloring. The staining of bacteria aims to make them sharply different from the background, which allows explore under microscope them morphology and structure. AT microbiology use simple and complex methods coloring drugs. At A SIMPLE METHOD of coloring, a smear is stained with any one dye, for example, water magenta (2-3 min.) or methylene blue (2-3 min.), washed water, dry up and microscopic.

5. Technics microscopy

Due to the very small size of bacteria, the study of their morphology is possible only at big magnification, achieved at help immersion oils, which allows you to create a single system between the glass slide and a special, x 90-fold (with black stripe) lens.

At microscopy painted objects necessary create bright lighting With help concave mirror, raised condenser and fully open diaphragm.

A drop of immersion oil is applied to the area of the smear on the glass lying on the table. The glass is then transferred to the microscope stage. The immersion lens is immersed in oil carefully, under the control of the eye, until there is obvious contact between the lens and the oil. Then the lens raise, not bringing out from drops oils and looking in eyepiece for finding object research ("fields vision"). Clear image drug achieved regulation first with a macro screw (for detecting an object), and then with a micro screw for adjustment sharpness Images.

6. Morphology major forms bacteria

known four main forms bacteria:

7. **Cocci are** microbes of a rounded shape, having a diameter of 1-2c. They differ between yourself on mutual location individual cells, which depends from way them division. If on graduation division cocci are separated on individual balloons, are obtained single cells cocci - Micrococcus.

8. Group from two cocci wears title diplococcus -Diplococcus (meningococcus, gonococcus have resemblance With beans, and lanceolate shape — Pneumococcus).

9. If the division of cocci occurs in only one direction and the resulting cocci do not are separated, then a thread of balls is obtained in the form of a chain, more or less long in dependencies from numbers cocci- Streptococcus.

10. When dividing in two mutually perpendicular directions, combinations arise along four cocci-Tetracoccus.

11. If division occurs in three mutually perpendicular directions, cocci connect in in the form of packages (cubes) and receive title — Sarcina.

12. Sharing in various directions without special correctness cocci form disorderly clusters cells,

reminiscent of grape bunches, why they and received title *Staphylococcus*.

Rod-shaped microorganisms are represented by the most numerous and diverse a group of bacteria. In the classification of rod-shaped forms, it is customary to refer to bacilli and clostridia are those sticks that are able to form spores, and those that are incapable of spore formation called bacteria. rod-shaped forms differ on size, location — one by one, couples, chain, disorderly and under angle. outline ends — rounded, chopped off, thickened, pointed.

Convolute forms - spirilla and spirochetes having the appearance of corkscrew-shaped convoluted cells. Pathogenic spirillums include the causative agent of sodoku (rat bite disease). To the twisted same relate campylobacter, having curves How at flying wings seagulls.

Spirochetes — thin, long, curved (spiral forms) bacteria, different from spirilla mobility, conditioned flexion changes cells. Spirochetes presented three childbirth pathogenic for person: *Treponema*, *Borrelia*, *Leptospira*.

Methods diagnostics infectious diseases

3. *Microscopic method* is in cooking drugs (native or painted simple or complex methods) from researched material and them microscopy With application various species microscopic technology (light, dark-field, phase-contrast, electronic). In bacteriology, the microscopic method called bacterioscopic in virology — viroscopy.

4. *cultural method* is in sowing researched material on artificial nutrient media for the purpose of isolating and identifying a pure culture pathogens. AT bacteriology cultural method received title bacteriological, a in virology - virological.

6. *The biological method* (experimental or bioassay) is to infect researched material sensitive or others biological objects (chicken embryos, cell cultures). It is used to isolate a pure culture of the pathogen, determining the type of toxin, determining the activity of antimicrobial chemotherapeutic drugs.

7. *Serological method* - consists in determining the titer of antibodies in the blood serum the patient, less often - in the detection of microbial antigen in the test material. With this goal are used reactions immunity.

8. *Allergic method* is in identifying infectious allergies (GZT) on diagnostic microbial a drug — allergen. FROM this goal put skin allergic tests With relevant allergens.

An object study medical microbiological laboratories — pathogenic biological agents — pathogenic for human microorganisms (viruses, bacteria, mushrooms, protozoa). AT accordance With types microorganisms allocate: bacteriological, virological, mycological, protozoological laboratories. Regulation conditions work With pathogens infectious diseases produced in according to the degree of danger of microorganisms to humans. Allocate four groups pathogens.

Group 1: pathogens of especially dangerous infections: plague, natural smallpox, fever Lassa, Ebola.

Group 2: exciters highly contagious bacterial fungal and viral infections: Siberian ulcer, cholera, fever, loose typhus, rabies.

Group 3: pathogens bacterial fungal, viral and protozoan nosological forms (whooping cough, malaria, polio, leishmaniasis).

Group 4: causative agents of bacterial, viral, fungal diseases (pseudomonal infection, amoebiasis, aspergillosis).

Microbiological laboratories work With PBA With pathogens especially dangerous infections (group 1 and 2). The special mode is maximally isolated by the individual and public risk.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.

5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION No. 2.

**Theme: Morphology microbes. Microscopic method research.
Control occupation.**

Educational goal:

1. Explore individual structures prokaryotic cells.
2. master complex methods coloring microbes.

Plan lessons:

1. Structure and chemical composition of a bacterial cell.
2. Features buildings bacterial cells.
3. Complex methods coloring, goals them use (methods Grama, Tsilya-Nelsen, Ozheshko, Ginsa Burri, Neisser).
4. Demonstration painted drugs on methods Grama, Burri Gins, Neisser, Ozheshko, Ziel-Nielsen.
5. Studying mobility bacteria, cooking drugs "hanging" and "crushed" a drop.

Independent Job students

1. cook mixed smear from pure cultures staphylococcus and intestinal sticks. Coloring with water fuchsin.
2. cook mixed smear from clean culture staphylococcus and intestinal sticks. coloring by Gram.
3. cook drugs "hanging" and "crushed" a drop.
4. Decor protocol research.

EQUIPMENT

1. Set for bacteriological research: Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological
loop-8 pcs. Tray With stand - on
8pcs
glass slides spirit
lamp -8pcs
Bottle With physical solution -8pcs.
2. Paint set:
By Gram: gentian purple, rr Lugol, water magenta, ethyl alcohol-2 set.
By Tsilyu Nielsen: magenta Tsilya, methylene blue, five% sulfuric acid - 2set.
By Ozheshko: 1.5% hydrochloric acid, magenta Tsilya, methylene blue, five% sulfuric acid - 2 set.
By Neisser: blue Neisser, rr Lugol, vesuvin. - 2 set.
By Burri Guinsu: ink, water magenta, glass With polished edges - 2set.
3. For definitions mobility subject glass With holes and pokpochnyeglass. - four PC.,
petrolatum - four PC.
4. Tubes with the growth of S.aureus cultures -2
pcs. Tubes with crop growth E.coli - 2 pcs.
Smears from vaccines BCG, for coloring according to Tsil Nielsen - 2 PC.

culture spore-forming bacteria on oblique agar, for coloring on Ozheshko - 2 PC.

Culture of capsular bacteria on oblique agar, for staining according to Burri-Gins - 2 pcs.
culture diphtheroids on oblique agar, for coloring on Neisser – 2 PC.

culture mobile bacteria on broth - 4 things.

5. Microscopes - 8 pcs.

Immersion butter - four PC.

6. Demonstration micropreparations: sputum smears stained according to Ziehl - Nielsen, smears capsule bacteria, painted on Burri Guinsu, smears diphtheria bacillus, stained according to Neisser, smears from anthracoid, stained according to Ozheshko, smears from cultures of Escherichia coli and staphylococcus, stained Gram.

7. Demonstration mobility bacteria in dark field.

8. Tables.

INFORMATIONAL MATERIL ON THEME

Technics difficult methods coloring

Complex ways coloring include sequential drawing on a drug dyes that differ in chemical composition and color, mordants and differentiating substances. it allows previously differentiate microbes (*differential- diagnostic ways*) and reveal certain structures cells (*special ways*).

1. Way coloring according to Gram

Coloring on Gramu is important diagnostic sign identification bacteria. AT result coloring on Gramu Everybody bacteria share on two groups; gram- positive (blue colors) and gram negative (red colors).

Coloring technique according to the method Grama

1. Fixed smear put on bacteriological bridge and cover stripe filter paper impregnated with gentian solution -violet on a paper strip inflict water. Through 2 minutes stripe are removed.
2. Not flushing a drug water, inflict solution Lugol on 1 minute. Then solution drained.
3. A drug discolor alcohol 20-30 seconds (before discharge purple trickle paints).
4. A drug washed water.
5. coloring water magenta — 2 minutes.
6. A drug washed water.
7. Dry up on air or filtering paper.

2. Way coloring on Tsil-Nelsen

Applies for detection some germs, rich lipids (for example, pathogen tuberculosis, leprosy and etc.)

1. For staining use concentrated solution carbolic magenta Tsilya. FROM goal improvements penetration dye in cage a drug With imposed on him stripe filtering paper and dye warm up above flame burner three times before appearance pair.
2. Then drug discolor five% solution sulfuric acid, previously removing filtering paper.
3. washed water.
4. Finishing up methylene blue in flow 3-5 minutes.
5. A drug washed water.
6. Dry up on air or filtering paper.

Bleaching acid leads to loss dye acid-stable microbes, and they stained in blue Colour. Acid resistant microbes remain red.

3. Way coloring on Burri Guinsu

1. mixed drop culture capsule bacteria With drop carcasses on end subject glass. Then cook smear How usually his cook from drops blood.
2. Smear dry up on air and fix in flame burners.

3. For detection bacteria smear stain water fuchsin.

At this way coloring bacteria stained in red Colour, a unpainted capsules stand out in contrast How bezel on black-brown background around bacteria.

4. Way coloring on method Neisser

1. On fixed smear apply blue Neisser 2-3 min.
2. Not rinsing with water inflict solution Lugol 10-30 sec.
3. Smear washed water.
4. Finishing up solution vesuvina — 1 min.

In the culture of yeast-like fungi, there are many grains of volutin. They represent connections, having, in difference from cytoplasm, alkaline reaction and because stained in dark blue Colour. Cytoplasm cells, possessing sour reaction perceives alkaline dye vesuvine and stained in yellow Colour.

5. Way coloring on method Ozheshko

1. On unfixed smear inflict 0.5% solution HCl and warm up on flame burner in flow 2 min. Before appearance vapors.
2. A drug washed water, dry up and fix.
3. Finishing up on method Tsilya-Nelsen.

controversy bacteria after given coloring acquire red Colour, a body bacteria-blue.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL LESSON #3

Topic: The influence of physical environmental factors on vitality microbes.

Educational goal:

1. Explore methods sterilization (physical, mechanical, chemical). 2. Explore methods control efficiency sterilization.

Plan lessons:

9. Methods sterilization: physical, chemical, biological, mechanical.
10. Device and application ovens Pasteur, autoclave, apparatus Koch.
11. Sterilization various medicinal funds in dependencies from them nature, forms, lability to physical factor.
12. Control quality sterilization.
13. concept about asepsis, antiseptic and disinfection.
14. Antiseptics and disinfectants.
15. Principles control quality disinfection.
16. Demonstration antiseptic and disinfectants funds.

Independent Job students:

1. Spend and take into account the results of the experiment to determine the effect of high temperature (80°C) to spore-forming (anthracoid) and asporogenic (E. coli and staphylococcus aureus) microorganisms.

- Fill protocol on form:

Accounting growth culture	Staphylococcus aureus	intestinal wand	Anthracoid
before warming up			
after warming up			

Vegetative forms of pathogenic microorganisms die at 50-60 °C for 30 minutes, and at a temperature of 70 °C for 5-10 minutes. Bacterial spores are more sustainability to high temperatures what explained content in them water in related state, big content salts calcium, lipids and density, layering shells. Consequently, staphylococcus aureus and intestinal wand after warming up are dying a disputes anthracoid survive. it and necessary take account of in evaluation results sowing.

- Fill on one's own table:

No.	Way sterilization	Apparatus	Reliability	sterilizable material
1.	Sterilization in flames			
2.	Plasma			

	Sterilization			
3.	Dry heat			
four.	Ferry under pressure			
five.	flowing ferry			
6.	Tyndalization			
7.	Filtration			
8.	Physical factors (UFL, gamma rays, ultrasound)			
nine.	Gas sterilization			
10.	Pasteurization			

EQUIPMENT

1. Set for microbiological research: Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological
loop-8 pcs. Tray With stand - on
8pcs
glass slidesspirit
lamp -8pcs
Bottle with physical solution - 8pcs
2. **For experience:**
Test tube with a suspension of Escherichia coli - 2
pcs. test tube With suspension staphylococcus- 2
pcs.
Test tube with suspension of anthracoid -
2 pcs. Water bath -1 PC.
Sterile bevelled agars -12 PC.
3. Demonstration: ovens Pasteur autoclave, apparatus Koch.
4. Demonstration antiseptic and disinfectants funds.
5. **Tables.**

INFORMATION MATERIAL ON THE TOPIC OF THE LESSON

STERILIZATION

Sterilization is deposition, t. e. complete release objects environmental environments from microorganisms and them dispute.

Sterilization produce various ways:

5. **Physical** (exposure to high temperature, UV rays, high pressure, pair, gamma rays, ultrasound).

6. **Chemical** (usage various disinfectants, antiseptics).

7. **biological** (application antibiotics).

8. **Mechanical** (filtration).

AT laboratory practice usually apply physical ways sterilization.

The possibility and feasibility of using one or another method of sterilization conditioned features material, subject sterilization, his physical and chemical properties.

To *physical* ways sterilization can attributed calcination in flame, sterilization dry heat in ovens Pasteur boiling, sterilization fluid ferry in apparatus Koch, ferry under pressure in autoclave, tindalization, pasteurization sterilization UFL, ultrasound.

Mechanical sterilization is carried out by filtration using bacterial filters made from various finely porous materials, the pores of the filters should to be enough small to ensure mechanical delay bacteria. This method sterilize nutritious environment, containing protein, serum, antibiotics; separate bacteria from viruses, phages, exotoxins.

In microbiological practice, Seitz asbestos filters, membrane filters (candles) Chamberlain and Berkefeld.

a) *Seitz filters* - discs made from a mixture of asbestos with cellulose, their thickness is 3-5 mm, diameter 35-140mm;

b) *membrane* filters - made of nitrocellulose, 0.1 mm thick and 35 mm in diameter. AT dependencies from size since designate 1,2,3,4,5;

c) *candles Chamberlain and Berkefeld* — hollow cylinders, closed With one the end cook them from kaolin with admixture of sand and quartz.

Chemical ways sterilization apply limited but they serve for warnings bacterial pollution nutritional Wednesdays and immunobiological drugs (vaccines and sera).

biological sterilization founded on application antibiotics, sometimes phages.

Disinfection - the use of chemicals (phenol, lysol, chloramine, peroxide hydrogen, sublimate, alcohol, etc.) to kill pathogenic bacteria in waste pathological material.

Systematization of devices, processing processes and means for disinfection and sterilization

Classification tools	Main types tools	Character processing and kinds impacts
critical - penetrate in sterilefabrics or vessels	All invasive surgical tools that havecontact with blood-supplied fabrics, scalpels, needles syringes, implant-you, burs, root needles, excavators, probes , trowels.	Sterilization - virucidal, sporicidal, tuberculocidal,bactericidal impact. long exposure: gamma- rays, plasma, continuous gas and chemical sterilization, autoclaving (2 atm. 15 min), dry heat (Maxim. mode, 2 hours)
Semi -critical come into contact with mucous shells (perexcept for som edental tools, listed higher)	- flexible endoscopes, catheters, instruments similar to flexible endoscopes, mirrors, crowns, tips turbines, a same prints(casts) teeth.	High level disinfection - virucidal, sporicidal, tuberculocidal, bactericidal effects. short-term exposure: gamma rays, plasma, short-term gas and chemical sterilization, autoclaving (1-1.5 atm. 15 min), dry heC.

	Thermometers for measuring temperature mucous shells, baths for hydrotherapy.	Medium level disinfection: virucidal, tuberculocidal, bactericidal impact.
	Ultrasonic baths and UV lamps dentists physiotherapy tools, spoons casts.	Means for chemical disinfection with indication of tuberculocidal labeling activity.
Non-critical come into contact with intact skin	Thermometers for measuring temperature skin covers, stethoscopes, cuffs devices for measurements pressure, desktop appliances and t. P.	Disinfection level: bactericidal effects. Funds for chemical disinfection without instructions on presence marking tuberculocidal activity.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and exercise on house ----- 10 min.

PRACTICAL EXERCISE No. 4.

Theme: Physiology microbes. Bacteriological method research.

Educational goal:

1. master bacteriological method diagnostics infectious diseases.
2. To study the types of nutrition of bacteria, the principles of cultivation microorganisms, classification nutritional avg.
3. Explore methodology receiving pure cultures of bacteria from researched material.

Plan lessons:

7. Nutrition bacteria: types, mechanisms receipts nutritional substances in microbial cell.
8. Principles cultivation microorganisms.
9. Bacteriological method diagnostics infectious diseases.
10. Nutrients environments: requirements, presented to nutritious Wednesdays; classification, composition, cooking.
11. Demonstration nutritional avg.
12. Inoculation of the test material (suspensions of microorganisms) on MPA by the method Drygalsky (1 stage).

Independent Job students:

3. Sowing researched material on method Drygalsky.
4. Familiarization With cooking nutritional avg.

EQUIPMENT

1. Set for bacteriological research Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological
loop-8 pcs. Tray With stand - on
8pcs
glass slides spirit
lamp -8pcs
Bottle with physical
solution - 8 pcs.
2. Sowing on Drygalsky:
cups With MPA 3 pcs.-4 set
Test tubes with a suspension of
microorganisms - 4 pcs. Sterile spatulas - four
PC.
3. Demonstration: nutrient media: MPA, KA, Endo medium, Kitt medium
Tarozzi, agar-agar, environments gissa, JSA, SCHA, Wednesday bismuth
sulfite, MPB.

4. tables

INFORMATIONAL MATERIL ON THEME

Microbiological research held for the purpose of highlighting pure cultures microorganisms, cultivation and study them properties. It necessary at diagnostics infectious diseases, for definitions specific accessories germs, in research work, for receiving products vital activity microbes (toxins, antibiotics, vaccines and t. P.). For cultivation microorganisms in artificial conditions necessary special substrates — nutritious environment. They are are basis microbiological work and define results Total research. environments must create optimal terms for vital activity microbes.

REQUIREMENTS PRESENT To WEDNESDAY:

8. Must be nutritious, i.e. contain in an easily digestible form all substances necessary for satisfaction food and energy needs microorganisms.

9. Have an optimal concentration of hydrogen ions. 10. Be isotonic for microbial cells.

11. Be sterile. 12. Be wet.

13. Possess a certain redox potential. 14. Be on opportunities unified.

Need in nutritional substances and properties environments at different species microorganisms are not the same. This eliminates the possibility of creating a universal environment. Besides, on choice toy or different environments affect goals research.

Group classification	Class	Examples
By composition	Simple	<i>Liquid - MPB, peptone water Dense — MPA</i>
	Complex	<i>Liquid — sugar bouillon Dense —</i>

		<i>sugar agar, blood agar</i>
By origin	natural	<i>Milk, folded serum, slice raw potatoes</i>
	artificial	<i>Milky salt agar Whey agar Ascites agar Blood agar</i>
	Synthetic	<i>Wednesday Needle, Wednesday 199</i>
By appointment	selective (elective) -for staphylococcus: - for gram (-) cocci anddiphtheroids: -for enterobacteria: - for cholera vibrio: -for lactobacilli and mushrooms Differential- diagnostic Universal enrichment media Preservative	<i>Milk-salt agar, bile-saltagar Serum media Salt mediatellurium Media with bile salts Peptone broth and alkaline agar tomato agar, rice agar, agar Saburo</i> <i>endo, Ploskireva, Levin, Ressel, gissaMPB, MPA, blood agar Wednesday Muller environments With glycerin</i>
By consistency	Liquid semi-liquid Dense	<i>MPB, peptone water, sugar MPB MPJele, gelatinous MPA, blood agar</i>

TIMELINE

1. Definition original level knowledge -----30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #5

Theme: Physiology microbes. Selection pure cultures aerobes and anaerobes.

Educational goal:

1. master methods allocation pure cultures aerobes.
2. Explore types breathing bacteria, ways creation conditions anaerobiosis.
3. master methods allocation pure cultures anaerobes.

Plan lessons:

11. Types breathing bacteria.
12. Ways creation conditions anaerobiosis.
13. Methods allocation clean aerobic cultures and anaerobes.
14. Sowing soil talkers for Wednesday Kitta Tarozzi.
15. Nutrients environments for anaerobes, methods cultivation and selection cleanculture anaerobes.
16. Studying cultural properties bacteria.
17. Studying colonies, grown on cups, sown Drygalsky's method .
18. Sowing microorganisms from studied colonies on oblique agar for receivingclean culture (2 stage).
19. Demonstration pigment formation bacteria.
20. Demonstration character growth bacteria on dense and liquid nutritional environments.

Independent Job students

1. Completion 1st stage bacteriological method. Studying cultural propertiesbacteria.
2. From grown colonies on MPA cook smear, paint on Gram.
3. Sowing from researched isolated colonies on oblique agar foraccumulation clean culture.
4. Demonstration of technology anaerobic cultivation and Wednesdays for anaerobes: high column of agar, Kitt-Tarozzi medium, thioglycol, Stuart. Demonstration microaerostC. Ways: Fortner, Weinberg.

EQUIPMENT

1. Swab kit: Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological
loop-8 pcs. Tray With stand - on
8pcs
glass slidesspirit
lamp -8pcs
Bottle With physical solution -8pcs.
2. Kit colors: By
Gram- 8 PC.
3. microscopes - 8 PC.

- Immersion butter - four PC.
4. 3 cups with colony growth on MPA-4 pcs.
 5. Slanted agar for reseeded - 8 PC.
 6. Demonstration of anaerobic culture techniques and media for anaerobes: high column of agar, Kitt-Tarozzi medium, thioglycol, Stuart.
 7. Demonstration microaerophilic. 8. Demonstration pigment formation bacteria.
 9. Demonstration of cultural properties on liquid and solid nutritional environments.
 8. Tables.

INFORMATIONAL MATERIAL ON THEME

Breath bacteria. Classification bacteria on type breathing.

The essence of respiration in microorganisms is the receipt of energy generated in the process direct biological oxidation substances oxygen or through dehydrogenation substrate. The accumulation of energy occurs in special structures of bacteria called mesosomes.

AT According to needs for oxygen bacteria subdivide on the following main groups:

1. obligate (strict) aerobes- microorganisms, which are growing and multiply only in presence oxygen. For example: *Vibrio cholerae* *Pseudomonas aeruginosa*.
5. Obligate anaerobes are microorganisms that grow and reproduce only without oxygen access. For example: *Clostridium botulinum*, *Clostridium tetani*.
6. Facultative anaerobes - microorganisms that can grow and multiply both in the presence of oxygen and in anoxic conditions. For example: *Escherichia coli*, *Salmonella typhi*.
7. Microaerophilic bacteria - microorganisms that grow and reproduce better at elevated content CO_2 and low content oxygen. For example: *Helicobacter pylori*, *Campylobacter coli*.

Culture methods anaerobes

Ways creation anaerobic conditions a) mechanical - removal (pumping out) air from anaerostat With help vacuum suction. Then anaerostat fill gas mixture which includes from 80% nitrogen, 10% hydrogen and 10% carbon dioxide gas;

b) chemical - the absorption of oxygen due to chemicals (alkaline solution pyrogallol, bicarbonate soda);

c) biological (method Fortner) — a joint cultivation anaerobes and aerobes. At the same time, one Petri dish with a dense nutrient medium (more often used Zeissler's medium) are sown with a culture of anaerobes, on the other - a culture of aerobes capable of absorb oxygen vigorously. The miraculous stick culture is used as aerobes (*Serratia marcescens*). The edges cups petri paraffin;

D) physical and chemical — sowing researched material on special environments for anaerobes, for example, Kitt-Tarozzi and Wilson-Blair media (iron sulfite agar). environments front sowing regenerate (boil on water bath in flow 15 minutes) for removal oxygen.

Composition environments Kitt-Tarozzi:

- pieces liver — for adsorption oxygen;
- 1% glucose - for implementation anaerobic glycolysis;
- semi-liquid agar - not admits oxygen in thickness environment.

Receipt pure culture anaerobes

1. Method Weinberg (method of dilution)

For receiving isolated colonies anaerobes from environments Kitt-Tarozzi With growth anaerobic bacteria take away culture Pasteur pipette With soldered end and sequentially lower this pipette first into 3 test tubes with saline, a then — 3 test tubes With melted semi-liquid sugar MPA. After temperature control at $37^{\circ}C$ in recent observed height isolated colonies anaerobes.

2. Method Peretz.

One of the last Weinberg dilutions in semi-liquid agar is poured into the lid cups petri and close her bottom So, to delete air. The edges cups petri paraffin. Sowing researched material on Wednesday Zeissler sectors With subsequent cultivation in anaerostat.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #6

Theme: Physiology microbes. Principles cultivation and identification microbes.

Educational goal: master bacteriological method diagnostics infectious diseases.

Plan lessons:

6. enzymes, classification. enzymes, splitting carbohydrates, proteins, fats, pathogenicity enzymes. Culture media used for study enzymatic activities microorganisms.
7. Examination purity dedicated culture macro and microscopically.
8. Identification of a pure culture of bacteria by morphological, tinctorial, cultural, biochemical, phagolyzable properties (3 stage).
9. bacteriophages, their application for identification bacteria.
10. Definitions antibiotic sensitivity method standard disks.

Independent Job

7. Cooking smear, coloring on Gram.
8. Sowing culture on Hiss environments and MPB.
9. Definition antibiotic sensitivity.
10. Demonstration of nutrient media for the study of enzymatic activity microorganisms.
11. Demonstration phenomenon bacteriophage on dense and liquid nutritional environments.
12. Demonstration of pure culture isolation of motile microorganisms on method Shukevich.

EQUIPMENT

1. Swab kit: Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological
loop-8 pcs. Tray With stand - on
8pcs
glass slides spirit
lamp -8pcs
Bottle With physical solution -8pcs.
2. Kit colors: By
Gram- 8 PC.
3. Microscopes - 8 pcs.
Immersion butter - four PC.
4. test tubes With growth on a beveled agar - 8 PC.
5. environments gissa (With glucose lactose mannitol, maltose sucrose), for sowing co

- oblique agar- four set.
6. test tubes With BCH for sowing co oblique agar, for study proteolytic properties - four PC.
 7. indicator papers on hydrogen sulfide and indole -four PC.
 8. cups With MPA for sowing co oblique agar on antibiotic sensitivity -four PC.
 9. indicator standard paper discs With antibiotics - four set.
 10. Sterile putty knife - four PC.
 11. Demonstration phenomenon bacteriophage on dense and liquid nutritional environments.
 12. Demonstration of pure culture isolation of motile microorganisms on method Shukevich.
 13. Tables.

INFORMATIONAL MATERIAL ON THIS TOPIC

III stage of the study. After 24 hours, a homogeneous growth was found on the beveled MPA. in the form of a continuous yellowish coating. In order to check the purity of culture isolation a smear is prepared from the test tube, stained according to Gram and microscopied. For rate purity culture necessary browse not less 10 fields vision. Finding insmears co oblique MPA only grape-like located gram-positive cocci, testifies about purity allocated cultures.

For the determination of biochemical bacteria produce sowing allocated culture in color series media (glucose, lactose, mannitol, sucrose, maltose and in the MPB for determining indole and hydrogen sulfide), or determine these properties using the Indicator System Paper disks (NIB).

Crops placed in thermostat at temperature 37°C on 24 hours.

Enzymes bacteria

Enzymes are highly specific biological catalysts, without which impossible life and reproduction. big number reactions, ongoing at life bacterial cells, indicates on Existence at bacteria significant quantities enzymes. Enzymes — substances protein nature with high molecular weight. Some of them are proteins other are complex proteins. They are built from two parts squirrel and non-protein parts, called prosthetic group. AT composition her may enter vitamins. nucleotides, atoms gland and etc. Connection between protein part enzyme and the prosthetic group can be strong and fragile. In the presence of unstable bonds in solutions, dissociation of the enzyme occurs and, at the same time, it can be released free prosthetic group.

Easy dissociating irostetic groups enzymes called coenzymes. Usually enzymes subdivided on the following main groups:

1. Oxidoreductases. all enzymes that catalyze redox reactions.
2. Transferases. catalyzing the transfer of certain groups (for example, amino groups, phosphate leftovers and t. d.
3. hydrolases, splitting through hydrolysis GT or other connections; to this class relate same phosphatase and deampnase — enzymes, chipping off respectively by hydrolytic phosphate or ammonium groups from various organic connections.
4. Liase, enzymes, chipping off from substrates non-hydrolytic through certain groups (for example, CO₂, NgO, SH₂ and t. d.).
5. isomerases, catalytic intramolecular perestroika in substrate.
6. Ligases (synthetases) - a class of enzymes that catalyze the addition to each other friend of two molecules with simultaneous breaking of the pprophosphate bond in triphosphates (for example, generators FROM — O, FROM — N or FROM — S connections).

Most high enzymatic activity possess saprophytes; in lesser degree this is property expressed at pathogenic bacteria. Studying enzymes pathogenic bacteria is extremely important, because on the basis of definitions enzymatic activity microbes can differentiate various kinds and determine nature Togo or other pathogen diseases. Along With this enzymatic activity microbes defines pathogenesis and clinical picture infectious diseases.

Enzymes differentiate on exo- and endoenzymes. Exoenzymes stand out cell in external

Wednesday, carry out processes splitting macromolecular organic connections on more simple, available for assimilation.

Bacterial enzymes are divided into constitutive and inducible. To the first group includes those enzymes that are synthesized by a bacterial cell outside dependencies from Togo, on which environment bacterium is grown. Inducible enzymes are produced by this bacterium only in response to the action of a specific inductor, present in environment.

TIMELINE

- | | |
|---|---------------|
| 1. Definition original level knowledge | ----- 30 min. |
| 2. Independent Job | ----- 30 min. |
| 3. Examination protocols | ----- 10 min. |
| 4. Cleaning working places | ----- 10 min. |
| 5. Control final level of knowledge and task on house | ----- 10 min. |

PRACTICAL OCCUPATION #7

THEME: Principles cultivation and identification microbes (continuation)

Delivery of the module on the topic "Influence on microbes of physical and chemical factors. Physiology microbes. Principles cultivation and identification microbes."

Educational goal:

1. Explore methodology allocation pure cultures bacteria from researched material.

Plan lessons:

3. Selection clean culture aerobic and anaerobic bacteria (conclusion).
4. Change module.

Independent work of students: IV

stage research. Accounting biochemical properties.

glucose	lactose	mannitol	sucrose	maltose	indole	hydrogen sulfide
To	To	To	To	To	+	-

- to — acid

CONCLUSION: Staphelococcus spp. was isolated and identified from a mixture of bacteria. based morphological, tinctorial (gram positive, clustered cocci) and cultural (smooth convex colonies of golden color) and biochemical properties.

EQUIPMENT

1. environments gissa With sowing (With glucose lactose mannitol, maltose sucrose), for results accounting - four set.
2. test tubes With MPB, for accounting results proteolytic properties - four PC.
3. Cups with MPA to record the results of antibiotic susceptibility - 4 pcs.
4. Demonstration enzymatic activity on Wednesdays gissa intestinal sticks and staphylococci.

5. Demonstration of enzymatic activity on API systems intestinal sticks and staphylococci.
6. Demonstration of antibiotic susceptibility by serial method dilutions.
7. tables

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION No. 8.

Theme: General virology. Methods virology. bacteriophages and phage typing.

Educational goal:

1. Explore morphology and ultrastructure viruses.
2. Explore structure and morphology bacteriophages.

Plan lessons:

1. Peculiarities biology viruses .
2. Principles classification viruses.
3. Types interactions viruses With cell.
4. Morphology and structure of bacteriophages, their practical application in medicine.
5. Change module.

Independent Job students:

- Studying demonstrations phenomenon bacteriophage on dense and liquid nutritional environments.
- Studying demonstrations intracellular inclusions (body Babesha-Negri).

EQUIPMENT

1. Demonstration phage typing.
2. Demonstration phenomenon bacteriophage on liquid and tight
 - a. nutritional environments.
3. Demonstration Wednesdays for cultivation cultures fabrics: environments hanks, 199, Needle.
4. Demonstration ovoscope With chicken embryo.
5. Demonstration micropreparations: intranuclear inclusions at measles, bodies Pashen at smallpox, bodies Babesh –Negri at rabies.
6. Demo micropreparations: tissue culture, cytopathic
 - a. action virus, reaction hemadsorption.
7. Demonstration reactions hemagglutination.
8. Demonstration color samples.

INFORMATIONAL MATERIL ON THEME

VIRUSES

Viruses possess properties, not allowing apply for them study ordinary methods microbiological research.

Distinctive properties viruses:

1. smallest sizes, measurable thousandths shares micron - millimicrons - from 8-10 m before 300-400 m.
2. Filterability through special finely porous filters, not passing other microorganisms.
3. non-cellular structure.
4. Absolute parasitism, those. ability live and multiply only in alive cells.

The form viral particles It has some types:

6. rod-shaped
7. spherical (spherical)
8. Cuboid
9. Capitate (spermatozoa)
10. Filiform

Mature viral particles, called *virions*, have the following structure: the central part is a DNA or RNA molecule, which forms a *nucleoid*. Around located protective protein shell, called *capsid*, built from morphological units, called *capsomeres*. Some complex virions have external shell, called *supercapsid*.

For microbiological diagnostics viral infections in the present time apply three major methodical approach:

4. **Virological diagnostics** - founded on allocation from researched virus material and his subsequent identification.
5. **Serological diagnosis** - determination of specific immunological changes in body under action viruses (more often Total With help diagnosticums reveal in blood serum antiviral antibodies).
6. **Molecular biological diagnostics** - detection in clinical material fragments nucleic acids causative viruses With help probes (hybridization NK) or PCR.

Individual viruses larger than 200 m can be stained according to Romanovsky - Giemsa; smaller viruses (variola viruses) can only be detected using special ways processing.

bacteriophages differ on chemical structure, type nucleic acid, morphology and nature of interaction with bacteria. The size of bacterial viruses hundreds and thousands once smaller microbial cells.

Typical phage particle (virion) includes from heads and tail. Length tail usually in 2 - four times more diameter heads. AT head contained genetic material - single stranded or double stranded RNA or DNA With enzyme transcriptase in inactive state, surrounded by protein or lipoprotein shell - ***capsid***, preserving genome outside cells .

Nucleic acid and capsid together make up the nucleocapsid. Bacteriophages can have icosahedral capsid, assembled from sets copies one or two specific proteins. Usually angles are made up of pentamers squirrel, and the support of each side of hexamers of the same or similar protein. Moreover, phages can be spherical in shape, lemon-shaped or pleomorphic. Tail is yourself protein handset - continuation protein shells heads, in basis tail available ATPase, which regenerates energy for injections genetic material. Exist same bacteriophages short offshoot, not having process and filiform.

phages, How and Everybody viruses, are absolute intracellular parasites. Although they transfer all the information to start their own reproduction in the appropriate host, they lack mechanisms for energy production and ribosomes for synthesis squirrel. Some phages have several thousand bases in their genome, while the G phage, most large from sequenced phages, contains 480 000 steam grounds - twice more than the average for bacteria, although still not enough genes for the most important bacterial organelles like ribosomes.

A large number of isolated and studied bacteriophages determines the need them systematization. Classification viruses bacteria has undergone changes: based on characterization host virus, taken into account serological, morphological properties, a then structure and physical and chemical composition virion .

Currently, according to the International Classification and Nomenclature of Viruses bacteriophages, in depending on type nucleic acids share on DNA- and RNA- containing.

Based on their morphological characteristics, DNA-containing phages are classified into the following groups: families: myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Plasmaviridae, Corticoviridae, Fuselloviridae, tectiviridae, microviridae, Inoviridae Plectovirus and Inoviridae Inovirus.

RNA containing: cystoviridae, Leviviridae

By character interactions bacteriophage With bacterial cell distinguish virulent and moderate phages. Virulent phages may only increase in quantity through lytic cycle. Process interactions virulent bacteriophage with cell consists of several stages: adsorption bacteriophage on cage, penetration in cell, biosynthesis components phage and then assembly, exit bacteriophages from cells.

Initially bacteriophages attached to phage-specific receptors on surface of the bacterial cell. The tail of the phage with the help of enzymes located on it end (in mostly lysozyme), locally dissolves shell cells, shrinking and contained in head DNA injected in cell, at this protein shell bacteriophage remains outside. injected DNA causes complete perestroika metabolism cells: stops synthesis bacterial DNA, RNA and proteins. DNA bacteriophage starts be transcribed With help own enzyme transcriptase, which after hits in bacterial cage is activated. First, early and then late mRNAs are synthesized, which enter the ribosomes. host cells, where early (DNA polymerases, nucleases) and late (proteins) are synthesized capsid and tail process, enzymes lysozyme, ATPase and transcriptase) squirrels bacteriophage. replication DNA bacteriophage going on on semi-conservative mechanism and carried out With participation own DNA polymerases. After synthesis late proteins and completion replication DNA comes final process - maturation of phage particles or fusion of phage DNA with an envelope protein and formation mature infectious phage particles.

Duration this process maybe make up from several minutes before several hours. Then going on lysis cells, and released new mature bacteriophages. Sometimes the phage initiates a lysis cycle, which leads to cell lysis and release of new phages. Alternatively, the phage can initiate lysogenic cycle, at which he instead of replication reversible interacts With genetic system host cell, integrating in chromosome or remaining in form plasmids. So the way viral genome replicated synchronously With DNA host and division cells, and such a state of the phage is called a prophage. Bacterium, containing prophage, becomes lysogenic as long as, under certain conditions or spontaneously prophage not will be stimulated on implementation lysing cycle replication. Transition from lysogeny to lysis is called lysogenic induction or prophage induction. For induction phage is strongly influenced by the state of the host cell prior to induction, same How Availability nutritional substances and other terms, having place in moment induction. Poor growth conditions favor the lysogenic pathway, while good terms contribute lysing reactions.

Highly important property bacteriophages is them specificity: bacteriophages lyse cultures of a certain type, moreover, there are so-called typical bacteriophages, lysing options inside kind, although meet polyvalent bacteriophages, which parasitize in bacteria different types.

Viruses highlighted in separate "kingdom" - Viga. They are contain only one type nucleic acids, do not have a cellular structure, do not have independent metabolism substances being intracellular parasites reproduction viruses carried out disunited way.

By international classification all viruses subdivided according to type of nucleic acids on 2 subtype - RNA- and containing DNA. Further separation viruses is carried out on the basis of the size of the viruses, the type of symmetry in the formation of capsids, presence or lack of external shells and quantities contained in them capsomeres.

VIROLOGICAL METHOD RESEARCH is main and most authentic, allows highlight virus from researched material With subsequent hisidentification. In order to accumulate virus-containing material, chicken embryos and tissue cultures (artificially cultured cells of a particular tissue). Tissue cultures are maintained on natural (medium 27, Enders) and synthetic (Wednesday 199, Needle, Melnik-Riordan) nutritional environments cooked on basis solutions Hanks and Earl. cultivated they in regular test tubes cups carrel, test tubes Barsky.

Methodology infections chicken embryo

There are several ways to infect a chicken embryo. Most often the material injected into the allantoic and amniotic cavities, onto the chorionallantoic membrane and into yolk sac. Before infection, the egg shell above the air chamber is processed 70% alcohol, burned on a flame, smeared with 2% iodine tincture, rubbed again alcohol and burn.

When infected in the allantoic cavity in the shell above the air chamber (borders which is outlined in advance with a pencil when the eggs are translucent in an ovoscope) are done a small hole with scissors or a scalpel. tuberculin syringe injected 0.1-0.2 ml virus-containing material on depth 2-3 mm below borders air cameras. Puncture in shell poured molten paraffin. Opening infected embryos are produced at the time of maximum accumulation of the virus (after 48-72 hours of incubation at temperature 37 FROM) after processing shells alcohol and 2% with a solution of iodine, it is dissected and discarded, the shell membrane is carefully removed and consider the chorionallantoic membrane around the site of infection for the presence of foci defeats (hemorrhage, whitish foci defeats).

Classification cellular crops:

- **primary** receive directly from fabrics animal and human through destruction proteolytic enzymes (trypsin, collagenase) intercellular substances. Dissociated cells placed in a nutrient medium are able to attach to the surface of the culture vessel and multiply, forming a monolayer - a layer with a thickness of one cell. With the help of special reagents, cells can be removed from the surface of one vessel and transplant in another. Such manipulation called **passage**. Primary culture withstand not more 5-10 passages.

- **transplanted** (passage) cellular culture able withstand unlimited number of passages. They originate from tumor cells that have lost differentiation and not having restrictions growth.

- **semi-transplantable** (diploid) cultures are fibroblast-like cells that capable of rapid reproduction, withstand up to 30-60 passages and retain the originalkit chromosomes.

Viruses can reproduce only in the cells of a living organism. Due to this viruses are cultivated by infecting chick embryos or tissue cultures, and suckling animals.

Detection (indication) viruses *Virus*

detection in chick embryo 1. Death

2. The appearance of an odor upon

opening 3. Cloudiness liquids in cavities

four. Education sores and hemorrhages on shells

Biological method research is in contagion sensitive to virus animal researched material, studying clinical and pathoanatomical paintings diseases. AT framework this method are used various animals: monkeys, rabbits, guinea pigs, dogs, mice, rats. Ways infections: subdural, intracerebral, intranasal and other.

Ways detection virus in body laboratory animals differ in dependencies from kind animal and type virus.

Detection viruses in culture cells

Revealing on cytopathic action (CPD). JPC is yourself degenerative changes in cells that appear as a result of reproduction in them viruses.

Distinguish complete and partial degeneration cells monolayer.

With complete degeneration caused, for example, by polio viruses, Coxsackie and ECHO, cells of the monolayer undergo significant changes, more of them slough off co glass. Remaining single cells wrinkled

Partial degeneration has several varieties: 3 .By type cluster formation (adenoviruses);

4 .By type focal destruction (smallpox, flu);

3.By type symplast formation (measles, parotitis, parainfluenza, herpes, HIV).

proliferative type changes typical for some oncogenic viruses, transforming cells in malignant.

Intracellular inclusion formed at reproductions some viruses in cytoplasm and nucleus of cells (smallpox, rabies, influenza, herpes, etc.) They are found when microscopy after coloring

monolayer on Romanovsky - Giemse, a same at luminescent microscopy.

Salk color test. As a result of the vital activity of cells in a nutrient medium accumulate sour products. AT result this Colour incoming in composition environments indicator (phenol red) turns orange. When cell culture is infected cytopathogenic viruses such as enteroviruses or reoviruses, cell metabolism suppressed medium pH and her Colour not are changing (Wednesday remains red).

Reaction hemagglutination. AT basis this reactions lies ability viruses, containing hemagglutinin receptors, "glue" erythrocytes. If eat hemagglutinins - RGA+(umbrella), if No - RGA - (button).

Reaction hemadsorption. Mechanism similar With RGA.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION No. nine

**Theme: Basics genetics. Molecular biological method diagnostics.
Polymerase chain reaction, her varieties.**

Educational goal:

1. Explore ways transmission genetic information between bacteria: transduction, transformation and conjugation.
2. Explore basics biotechnology and genetic engineering.

Plan lessons:

1. Medical biotechnology.
2. Genetic recombination: transduction, conjugation, transformation
3. Role genetic recombinations in genetic engineering and medical biotechnology.
4. The use of plasmids in genetic engineering research. 5. Application genetic and molecular biological methods in diagnostics infectious diseases: PCR, method molecular probes. 6. Biopreparations, obtained by the method genetic engineering (vaccines, monoclonal antibodies, hormones, diagnostics).

Independent Job students

1. Accounting results experience transformations.
2. Accounting results experience transduction.
3. Accounting results experience conjugation.
4. Specify correct answers in test tasks. 5. Sketching tables.

EQUIPMENT

1. Accounting results experience transformations.
2. Accounting results experience transduction.
3. Accounting results experience conjugation.

INFORMATIONAL MATERIAL ON THEME LESSONS

Staging experience transformation

Recipient — strain *Bacillus subtilis* str (hay wand, sensitive to streptomycin); donor - DNA isolated from strain *B. Subtilis* Str (resistant to streptomycin). Selective Wednesday for selection recombinants (transformants) nutritious agar, containing one hundred U/ml streptomycin.

To 1 ml of *B. subtilis* broth culture add 1 µg/ml of DNase solution in 0.5 ml magnesium chloride solution to destroy DNA that has not penetrated into bacterial cells recipient strain, and incubated for 5 min. To determine the amount formed streptomycin-resistant recombinants (transformants) 0.1 ml undiluted mixture is sown on a selective medium in a Petri dish. For determining the number of cells of the recipient culture in isotonic sodium chloride solution prepare 10-fold dilutions up to 10^{-5} - 10^{-6} (to obtain a countable amount colonies), inoculated 0.1 ml on nutrient agar without streptomycin, and for control - on streptomycin agar. The recipient culture should not grow on the last medium, because the she sensitive to streptomycin. Sowing incubate at 37° FROM. On the next day, the results of the experiment are taken into account and the frequency of transformation is determined by the ratio of the number of grown recombinant cells to the number of recipient cellsstrain.

Let us assume that when seeding 0.1 ml of the culture of the recipient strain in a dilution of 10^{-5} has grown 170 colonies, a at sowing 0.1 ml undiluted mixtures — 68 colonies recombinant strain. Because the each the colony formed in result breeding only one bacterial cell, then in 0.1 ml sown culture recipient contained 170×10^5 viable cells, a in 1 ml — 170×10^6 , or 1.7×10^8 . At the same time, there are 68 recombinant cells in 0.1 ml of the mixture, and 680 in 1 ml. or 6.8×10^2 .

So way, the frequency transformation in this experience will be is equal to:

$$\frac{6.8 \cdot 10^{-2}}{1.7 \cdot 10^8} = 4.0 \cdot 10^{-6}$$

Staging experience specific transduction

Recipient — strain *E. coli* lac- , - deprived 3-galactosidase operon, controlling lactose fermentation. Transducing phage - phage X dgal, in the genome whom part genes replaced (3-galactosidase opera-ron *E. coli*. He is defective, t. e. not able call productive infection ending lysis intestinal sticks, and denoted letter d (phage dgal) With name contained in the genome of the bacterial operon gal. Selective environment - Wednesday Endo, on which lactose-negative bacteria of the recipient strain form colorless colony, a lactose-positive colonies recombinant strain acquire red Colour With metallic shade. To 1 ml 3 hour bouillon culture recipient strain add 1 ml of transducing phage dgal at a concentration of 10^6 - 10^7 particles in 1 ml. The mixture is incubated for 60 minutes at 37° C, after which a row is prepared 10-fold dilutions (depending on the expected concentration of bacteria) for obtaining a countable number of colonies. From a test tube with a dilution of 10^{-6} make inoculation of 0.1 ml of culture per 3 Petri dishes with Endo medium and evenly distribute liquid spatula on surfaces environment.

crops incubate in flow 1 days, after what note results experience and calculate frequency transduction on relation quantities cells recombinants (trans-ductants) found on all plates, to the number of cells of the recipient strain. For example, after inoculation of 0.1 ml of the mixed culture at a dilution of 10^{-6} on 3 plates of Endo medium grew 138, 170 and 160 colorless colonies of the recipient, respectively. strain, on the first and last cups - 5 and 1 colonies of red transductants. Consequently, transduction frequency in this case will is equal to:

$$\frac{(five + 1) \cdot 10 \cdot 10^{-6}}{(138 + 170 + 160) \cdot 10 \cdot 10^{-6}} = \frac{6}{48} = 1.3 \cdot 10^{-2}$$

Setting up a conjugation experiment with the aim of transferring a fragment of a

chromosome that contains gene leu, controlling synthesis leucine.

Donor - strain *E. coli* K12 hfr *leu*^S; recipient - strain *E. coli* K12F- - *leu*⁺ Str^R. hfr

— designation states, for whom characteristic high frequency recombination. Selective medium for the isolation of recombinants - minimal glucose-salt medium: KN₂RO₄ — 6.5 G, MgSO₄ — 0.1 G, (NH₄)₂SO₄ — 1 G, Ca(NO₃)₂ — 0.001 G, FeSO₄ — 0.0005 G, glucose — 2 G, streptomycin — 200 U/ml distilled water — 1 l.

To 2 ml of a 3-hour culture of the recipient, add 1 ml of the broth culture of the donor. The cultures are incubated at 37 °C for 30 minutes. Then the mixture is diluted to 10⁻²-10⁻³ and are sown in 0.1 ml per selective agar medium in Petri dishes, on which only recombinant colonies. As a control, donor seed is sown on the same medium. and recipient strains, which not will grow on her, t. to. the first strain sensitive to streptomycin, and the second auxotrophic leucine. In addition, culture donor strain sown on selective Wednesday without streptomycin, a culture recipient strain - on a complete medium (nutrient agar) with antibiotics for definitions numbers viable cells. crops incubate at 37 °C FROM before next day. After counting numbers grown colonies define frequency recombinations on relation quantities recombinant cells to recipient.

For example, after inoculation of 0.1 ml of a mixture of donor and recipient cultures in dilution 10⁻² grew 150 colonies of recombinants, and after inoculation of 0.1 ml of the culture of the recipient from dilutions 10⁻⁶ 75 colonies. So the way frequency recombination will be is equal to:

$$\frac{150 \cdot 10 \cdot 100}{75 \cdot 10 \cdot 10^6} = \frac{1.5 \cdot 10^5}{7.5 \cdot 10^8} = 2.0 \cdot 10^{-4}$$

Polymerase chain reaction (PCR) — one from contemporary molecular genetic methods, founded on principle multiple copying (amplifications) certain site DNA or RNA. AT result this process amount of determined DNA in sample increases in dozens million once, what does possible subsequent detection amplified DNA. So the way PCR allows you to identify negligible fragments of DNA characteristic of a particular species germs, and exactly to identify this view.

PCR in cage was open more thirty years old back Nobel laureates A. Kronberg and D.Ledeberg. Principle of the PCR method in vitro was developed by K.Mulis in 1983, same become Nobel laureate. Almost immediately appeared messages about his practical application. However in this period due to absence necessary PCR equipment was carried out by manually transferring the tubes to thermostats with the desired temperature. The enzyme DNA polymerase, necessary for DNA synthesis, was destroyed after everyone denaturation stage (at 95°C), so it needed to be constantly added new servings.

In 1988, a thermostable DNA polymerase was obtained from the bacterium *Thermophilus aquaticus*, living in hot springs. Were developed special appliances for amplifications (thermocyclers). Modern laser sequencing technologies created (deciphering the nucleotide sequences of DNA). This led to PCR becoming accessible for wide applications in laboratory practice.

AT the present time most quickly develop five major directions genetic diagnostics:

- infectious diseases (tuberculosis, gonorrhea, viral infections - hepatitis B and FROM, HIV CMV and etc.),
- oncological diseases,
- genetic diseases,
- identification personalities (transplantation bodies and fabrics, judicial medicine, definition paternity),
- diagnostics pathogens in food.

Test material: blood, serum, lavage masses, sputum, saliva, gastric the juice, biopsy material, strokes, flushes.

staging PCR includes the following stages:

1. Selection DNA (RNA) from researched material (sample preparation).

Cells lyse detergents or high temperature. Then separate DNA from cell debris and destroy cellular nucleases. All this provide appliances: minicentrifuges, developing speed 12000 -14000 rpm per minute, vortexes for mixing, mini thermostats for test tubes providing rapid change temperature from +30°C up to +100°C.

2. Immediate amplification allocated plots (copies) nucleic acids.

PCR provides fast and multiple multiplication, amplification (amplification - amplification, increase) in the number of genome fragments. For this, in a test tube with isolated DNA is added with the necessary reagents and placed in an amplifier (thermal cycler). This device allows cyclically change and support swings temperatures in test tube on several tens of degrees in a few seconds. If the test tube contains the desired DNA, then her going on a number of processes:

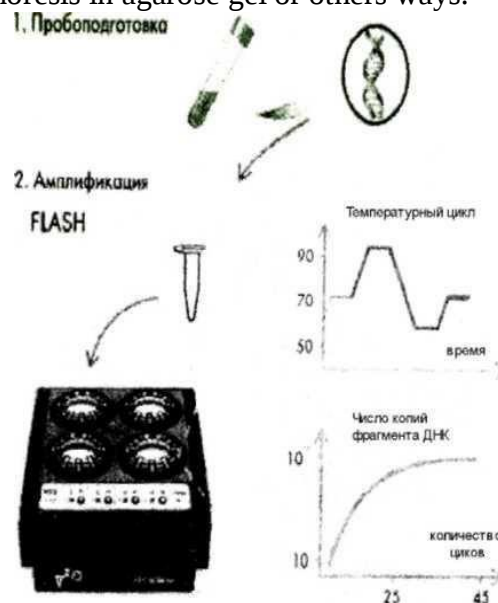
- As a result of heating to 94 -95 ° C, the double strand of DNA is divided into two separate chains.

- To single stranded target DNA joins primer.

Primer — this is sequence from 15 — thirty nucleotides, complementary marker DNA fragment. When creating the optimum temperature (45-70°C), binding (annealinD) of the primer to the corresponding DNA region: one primer on one threads, another — on second threads DNA. Annealing leaks in accordance With rule complementarity of Chargaff, meaning that in a double-stranded DNA molecule, opposite adenine is always thymine, a against guanine — cytosine.

- Synthesis (elongation) - completion of the second chainDNA.

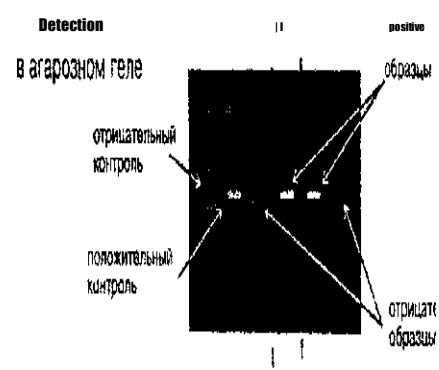
DNA polymerase adds nucleotides to primers, completing double-stranded DNA fragments (~ at 72°C). The newly synthesized DNA fragments serve as a template for synthesis of new chains in the next cycle of amplification - this is the chain reaction in PCR. As a result, the number of fragment copies increases exponentially and after 25 cycles of amplification, 10^6 copies of the fragment are synthesized. After 30 - 40 cycles synthesized such number DNA, which can visually take account of after electrophoresis in agarose gel or others ways.



3. Definition (detection) products PCR, received on second stage.

The identification of the accumulated product is most often carried out using electrophoresis in 2-3% agarose gel containing ethidium bromide (a specific fluorescent dye DNA). Absorbing UV

light dye, connected With DNA, fluoresces. AT



As a result, an orange stripe is visible at the level of the control DNA. In addition, they use enzyme hybridization method or real-time PCR using fluorescent dyes.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #10

Theme: Antibacterial chemotherapy

Educational goal:

3. Explore mechanism actions antibiotics on microbial cell.
4. To study the method for determining the sensitivity of bacteria to antibiotics.

Plan lessons:

9. antibiotics, definition, classification in chemical structure, spectrum types and mechanism actions.
10. Chemotherapeutic drugs, mechanism their actions on microbial cell.
11. Mechanisms medicinal sustainability bacteria.
12. Side effects of antibiotics and synthetic antimicrobial drugs.
13. Methods and units measurements antimicrobial activity.
14. Antiviral chemotherapy drugs.
15. Demonstration antibiotics With various mechanisms and spectro actions.
16. Change module.

Independent Job students

6. Take into account results disk antibiograms.
7. Take into account results cassette micromethod.
8. Design protocol research.

EQUIPMENT

1. cups petri With MPA With antibiotic sensitivity -four PC.
2. Demo: indicator paper discs With antibiotics.
3. Demonstration: method serial dilutions.
4. Demonstration: antibiotics various spectrum actions.
5. Tables.

INFORMATIONAL MATERIAL ON THEME OF THE LESSON

All antibiotics are selective. Their relative harmlessness for human determined, before Total, topics what they specifically suppress such metabolic processes in microbial cage or virus, which missing in eukaryotic cage or unavailable for them. AT this relation unique is mechanism actions beta-lactam antibiotics. targets for them are transpeptidases that complete the synthesis of cell wall peptidoglycan. Because the cellular wall eat only in prokaryotes, in the eukaryotic cage No targets

for beta- lactam antibiotics. Transpeptidase present yourself kit enzyme proteins, localized in the cytoplasmic membrane of the bacterial cell. Separate beta lactams differ in the degree of affinity for a particular enzyme, which received the name of penicillin-binding proteins. Therefore, the biological effect of beta-lactam antibiotics different: bacteriostatic, bactericidal, lytic.

Except beta-lactam antibiotics, synthesis cellular walls amaze such antibiotics like bacitracin, fosfomycin, cycloserine, vancomycin, ristomycin, however otherwise by, how penicillin. Everybody they, Besides cycloserine, cause bactericidal Effect.

Mechanism actions such antibiotics, How chloramphenicol, tetracyclines, streptomycin, aminoglycosides, erythromycin, oleandomycin, spiramycin and other macrolides, lincosamides, fusidic acid, is associated with inhibition of protein synthesis at the level ribosomes 70S. Although bacterial ribosomes 70S have the same structure in principle as ribosomes of 80S eukaryotic cells, their proteins and protein factors involved in the work protein-synthesizing systems, different from such ribosome 80S. This explained selectivity of action specified antibiotics on proteinaceous synthesis bacteria.

Different antibiotics differently block synthesis squirrel. Tetracyclines block binding at-RNA on A-section ribosomes 70S. Chloramphenicol suppresses peptidyl transferase reaction. Streptomycins impede transformation initiator complex into a functionally active ribosome. Erythromycin blocks reaction translocations. Puromycin, joining to growing end synthesized polypeptide chains, causes premature department her from ribosomes. Mechanism The action of fluoroquinolones is associated with their selective inhibition of bacterial enzymes. DNA gyrase, participating in replication DNA. Fluoroquinolones contact co specific plots DNA, which created impact DNA gyrase, and suppress her activity.

Rifampicins inhibit the activity of DNA-dependent RNA polymerases, as a result of which bacteria suppressed processes transcription.

Activity antitumor antibiotics connected with the what they or are inhibitor synthesis DNA (bruneomycin), or suppress activity DNA dependent RNA polymerase, t. e. blocks transcription (anthracyclines, actinomycins, olivomycin).

Accounting for the results of determining the sensitivity isolated from the test material microorganisms to antibiotics held next way: on working table there is a Petri dish on which the isolated from the studied material was sown microbe and were inflicted on equal to distance friend from friend discs With antibiotics (this method work outlined in practical guide).

The student must conclude the degree of sensitivity of the isolated culture to antibiotics. Meaning given research comes down to next: surface nutritional environments on cup moisten suspension dedicated clean culture in physical solution and so way achieved uniform distribution culture on all cup.

Disks with antibiotics are placed “on top” of the inoculation and the plates are incubated in a thermostC. FROM disks, impregnated every separate antibiotic going on diffusion antibiotics in thickness agar. How more sensitive culture to antibiotic topics smaller his efficiency concentration and topics more diameter zones delays growth culture around certain disk. At this result accounted for following scheme (table).

culture highly sensitive	diameter zones growth inhibition bacteria thirty and more mm.
culture medium sensitive	diameter zones oppression growth bacteria not less 20 mm.
culture weakly sensitive	diameter zones oppression growth bacteria not more 10 mm.

TIMELINE

1. Definition original level knowledge ----- 30 min.

2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #11

Subject: Symbiosis. Residents and pathogens. Fungi-causative agents of mycoses. Educational goal:

1. Explore stages and factors symbiosis human With microbes.
4. Study the microflora body human
5. Explore mushrooms - pathogens of mycoses and mycological method research

Plan lessons:

1. Stages and factors symbiosis human With microbes.
2. Terms formation associations residents.3.

Differences pathogens from residents.

4. What methods can study microflora human?
5. Composition resident microflora skin covers person.

Independent student work:

1. Cooking smears from yeast mushrooms, paint them simple method (methylene blue) and microscoping.
2. Cooking and microscopy native drugs from cultures moldy mushrooms.
3. View and sketch demonstration drugs:
 - b) actinomycetes, painted on Gram;
 - b native preparations from cultures of mold fungi (mucor, aspergillus, penicillium); in yeast mushrooms, painted methylene blue;
4. Sowing material With fingers hands per cup with MPA (method prints).
5. Sowing detachable from the nose and pharynx on MPA.

EQUIPMENT

1. Set for microbiological research: Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological loop-8 pcs. Tray With stand - on 8pcs
glass slides spirit lamp -8pcs
Bottle With physical solution -8pcs.
2. Kit colors: By Gram- 8 PC.
3. Microscopes - 8 pcs.
Immersion butter - four PC.
4. cups petri With MPA, separated on sectors for sowing prints fingers hand-4 things.
5. cups petri With ka, for sowing smear from pharynx - 4 pcs.
6. Demonstration bacterial preparations: eubiotics.
7. Tables.

INFORMATIONAL MATERIAL ON THEME

Microorganisms are in various relationships with each other. A joint The existence of two different organisms is called *symbiosis*. There are several options useful relationship: metabiosis, mutualism, commensalism, satelliteism.

Antagonistic relationship expressed in form unfavorable the impact of one type of microorganism on another, leading to damage and even death the last one. Forms antagonism: competition, predation, parasitism.

Microflora organism human

organism human inhabited approximately 500 types germs, constituents his normal microflora, in form communities microorganisms (*microbiocenosis*). They are are in able equilibrium (*eubiose*) friend With friend and organism person. Distinguish normal microflora various biotopes: skin, mucous shells cavities mouth, top respiratory ways, gastrointestinal tract and urogenital systems. AT body allocate permanent and transient microflora. *Permanent* microflora is represented microorganisms that are constantly present in the body. *The transient* microflora capable to long existence in body. Permanent microflora can divide on obligate and optional. obligate microflora (bifidobacteria, lactobacilli, peptostreptococcus, intestinal wand and etc.) is basis microbiocenosis, and facultative microflora (staphylococci, streptococci, Klebsiella, clostridia, some mushrooms and etc.) includes lesser part microbiocenosis. microorganisms, constituents normal microflora, concluded in highly hydrated exopolysaccharide nomycin matrix, forming biological film, resistant to various influences.

Protocol research

No.	researched material	results research	Graphic image

Mushrooms (fungi, mycetes) — heterogeneous group eukaryotic microorganisms. Mushrooms have nucleus With nuclear shell, cytoplasm With organelles cytoplasmic membrane (which contains phospholipids and sterols) and powerful cellular wall consisting of glucan, cellulose, chitin, protein, lipids, etc. Mushrooms consist of long thin threads (gif), intertwined in mycelium, or mycelium. gifs lower mushrooms — phycomycetes - do not have partitions. In higher fungi - eumycetes - hyphae are divided partitions; their mycelium multicellular. Mushrooms multiply disputes sexual and asexual ways, a also vegetative through (budding or hyphae fragmentation). Mushrooms, breeding sexual and asexual by, relate to perfect. Imperfect mushrooms are called mushrooms that do not have or have not yet described the sexual tract. breeding. Asexual reproduction is carried out in fungi using endogenous spores, maturing inside round structures — sporangia, and exogenous dispute — conidium, emerging on tips fruitful hyphae.

Fungi can be divided into 7 classes: chytridiomycetes, hyphochytridiomycetes, oomycetes, zygomycetes, ascomycetes, basidiomycetes, deuteromycetes. overwhelming majority fungi that cause diseases in humans (mycoses) are imperfect fungi. For diagnostics mycoses may to be used microscopic (cultural), allergic, serological, biological and histological methods research. Material for research may to be pus, sputum, affected hair, nails, skin flakes, bone marrow punctates, lymph nodes, internal organs, blood, bile, feces, tissue biopsy specimens, etc. Most often used for staining smears methods Grama, Ziel-Nielsen, Romanovsky-Giemsa

TIMELINE

- | | | |
|--|-------|-------------|
| 1. Definition original level knowledge | ----- | thirty min. |
| 2. Independent Job | ----- | thirty min. |
| 3. Examination protocols | ----- | 10 min. |

- | | | |
|--|-------|-------------|
| four. Cleaning working places | ----- | 10 min. |
| five. Control final level of knowledge and task on house | ----- | 10 minutes. |

PRACTICAL OCCUPATION #12

CHANGE MODULE ON TOPIC: "General virology. Basics genetics. Antibiotics. Symbiosis. Pathogenic mushrooms".

PRACTICAL OCCUPATION #13

Topic: Physiological mechanisms of immunity. Immune reactions (agglutinations and precipitation).

Educational goal:

1. will study physiological mechanisms immunity.
2. Explore serological methods laboratory diagnostics.

Plan lessons

1. Antigens them nature. Gaptens. Antigens bacteria.
2. Antibodies, classification. Structure immunoglobulins, main classes.
3. humoral and cellular immune response
4. Serological reactions, them essence and mechanism, practical application. Serodiagnostics. Seroidentification.
5. Agglutination reaction , staging methods , reaction phases , practical application.
6. Reaction precipitation, ways performances, practical application.
7. diagnosticums, classification, application.
8. Diagnostic serum, receiving and kinds diagnostic sera - agglutinating (adsorbed and non-adsorbed, mono- and polyvalent), precipitating.
9. Demonstration deployed reactions agglutination, reactions hemolysis.
10. staging reactions ring precipitation.
11. Demonstration diagnosticums and diagnostic sera.

Independent Job students:

1. staging and accounting indicative reactions agglutination on
2. glass slide to identify isolated pure culture _ Gram-negative sticks.
3. Statement and accounting of an extended agglutination reaction in order to serodiagnosis abdominal typhus.
4. Setting up and taking into account the reaction of thermoring precipitation in order to seroindication Siberian ulcers.

EQUIPMENT

1. Set for microbiological research: Tripod- 8 PC.
Tweezers - 8 pcs. Bacteriological loop-8 pcs. Tray With stand - on 8pcs
Subject glasses. spirit lamp -8pcs

- vials With physical r-rum.-8pcs.
2. Statement and accounting of the approximate reaction of agglutination to glass slide to identify isolated pure culture Gram-negative sticks:
 Tube with slant agar with E.coli growth – 4 pcs. Slide -4 things.
 test tube With polyvalent coli-serum -four PC.
 3. Statement and accounting of an extended agglutination reaction in order to serodiagnosis abdominal typhus.
 Researched serum-8pcs.
 Diagnosticum "O" -2 pcs.
 Diagnosticum "N" - 2 pcs.
 Diagnosticum "OH (A)" -2 pcs.
 Diagnosticum "HE IS IN)"- 2 pcs. test tubes clean 8pcs- 8 sets.
 Test tubes with physiological solution - 8 pcs.
 Sterile pipettes.
 4. Statement and accounting of the thermoring precipitation reaction in seroindication Siberian ulcers. Ascoli order to
 Test tube with immune serum - 4 pcs. Test tube with normal serum - 4 pcs. Test tube with physiological solution - 4 pcs. Precipitinogen -4 things.
 Pasteur pipettes - 4 pcs.
 5. Demonstration: reaction Vidal.
 6. Demonstration reactions precipitation in gel on Ouchterlony.
 7. Demonstration bacterial preparations: serum, diagnostics.
 8. Demonstration: RPGA.
 9. tables

INFORMATIONAL MATERIAL ON THEME LESSONS

Under **immunity** (from LC. *immunitas* — release, deliverance from something) in biology and medicine understand the complex of body reactions aimed at preserving its structural and functional integrity when exposed to the body genetically alien substances How incoming from the outside So and formed inside organism.

Distinguish some major species immunity:

-*Hereditary immunity* (congenital, species) conditioned development in process phylogenesis genetically fixed immunity kind to given antigen or microorganism.

-*Acquired immunity* specific and not transmitted on inheritance. He formed naturally and created artificially. Natural acquired immunity appears after an infectious disease (smallpox, measles, etc.). Artificial acquired immunity arises at vaccination.

Immunity it happens *active* and *passive*. *Active immunity* produced organism in result impact antigen on immune system (for example, at vaccination). *Passive immunity* is caused by antibodies transmitted from the immune system. mothers to kid at birth or through introductions immune serums, a same at transplantation of immune cells.

Active immunity can be *humoral* (caused by antibodies), *cellular* (conditioned immunocompetent cells) and *cellular humoral* (conditioned and antibodies, and immunocompetent cells). For example, antitoxic immunity to botulism and tetanus is humoral So How he conditioned antibodies circulating in the blood immunity to leprosy or tuberculosis is cellular, and to smallpox - cellular humoral.

sterile immunity , which persists in the absence of a microorganism, and *non-sterile*, which

exist only at availability pathogen in body. Classic example non-sterile immunity is immunity at tuberculosis.

Separately allocate So called *local immunity*, which protects individual plots organism, for example, mucous shells from pathogens infectious diseases. It is formed with the participation of secretory immunoglobulin A and characterized more active phagocytosis.

Antigens — this is any genetically alien for given organism substances (usually biopolymers), which, having entered the internal environment of the body or formed in body, cause reciprocal specific immunological reaction: synthesis antibodies, the appearance of sensitized lymphocytes or the development of tolerance to this substance hypersensitivity delayed or immediate types, immunological memory.

Antigens possess specificity which tied With certain chemical group in composition molecules, called determinant or epitope. determinants antigen are those parts of it that are recognized by antibodies and immunocompetent cells.

Distinguish between *complete* and *defective (haptens) antigens*. The antigens that cause full-fledged immune answer, having 2 and more determinants, called *complete*. it organic substances microbial, vegetable and animal origin. *Haptens* can be small molecular weight chemicals or more complex chemical substances not possessing properties full-fledged antigen: some bacterial polysaccharides, polypeptide tuberculosis sticks (RRD), DNA, RNA, lipids, peptides. *Haptens* due to small molecular masses not are fixed by immunocompetent cells of the macroorganism and cannot cause a response immunological reaction. *Semi-haptens* — inorganic radicals (iodine, bromine, nitro group, nitrogen and etc.), joined to protein molecule, may change immunological specificity squirrel.

Antibody formation. In response to the introduction of an antigen, the immune system produces antibodies — proteins, capable specifically unite With antigen, caused them education and, so the way participate in immunological reactions. Relate antibodies to y-globulins, i.e., the least mobile fraction of proteins in an electric field blood serum. In the body, y-globulins are produced by special cells - plasma mocytes. In accordance with the International Classification of y-globulins that carry the functions antibodies are called immunoglobulins and are denoted by the symbol Ig. Consequently, antibodies — this is immunoglobulins, produced in answer on introduction antigen and capable of specifically to interact With this same antigen.

Functions of antibodies. The primary function of antibodies is the interaction of their active centers with complementary determinants of antigens. Secondary function of antibodies includes from them capabilities:

- bind antigen With goal his neutralization and elimination from organism;
- participate in recognition "foreign" antigen;
- provide cooperation immunocompetent cells (macrophages, T- and AT- lymphocytes);
- participate in various forms of the immune response (phagocytosis, killer function, immunological tolerance, immunological memory, hypersensitivity immediate type, hypersensitivity delayed type).

Proteins of immunoglobulins are chemically classified as glycoproteins, since consist from protein and sugars; built from eighteen amino acids. Distinguish five classes immunoglobulins: IqM, IgG, IgA, IgE, IgD. Immunoglobulins M, G, A have subclasses. For example, IgG It has four subclass (IgG1, IgG2, IgG3, IgG4).

Immunological memory called ability organism at repeated meetingwith the same antigen to react more actively and more rapidly immunity those. to react on type secondary immune response.

Immunological tolerance phenomenon opposite immunological memory. In this case, in response to the repeated introduction of the antigen, the body, instead of vigorous workings immunity shows unreactivity, not answers immune reaction t. e. tolerant antigen.

I. Agglutination reaction on subject glass

Apply on a glass slide at a sufficient distance friend from three friends drops: physiological solution, typhoid agglutinating serum (No. 1) and dysenteric agglutinating serum (No. 2). Add the culture under study to the drop physiological solution and thoroughly grind in her before appearance expressed turbidity. Transfer the prepared suspension with a bacterial loop into serum

No. 1 and thoroughly mix. Further bacteriological loop necessary sterilize calcination. Then take the bacterial loop material from the culture suspension in a drop physiological saline and add it to a drop of serum No. 2. Glass lightly and carefully shake to thoroughly mix. Accounting for the results of the reaction is carried out after 1-2 minutes: uniform turbidity remains in a drop of saline, then How in drop one from sera celebrated agglutination. Signs agglutination are: dropping out grains agglutinate and enlightenment liquids. AT case detection in control drop With physiological solution spontaneous agglutination results reactions are not subject to further accounting, a herself reaction requires repeated staging.

II. *deployed reaction agglutination*

deployed agglutination reaction was set to determine the antibody titer in the patient's blood serum.

The test serum is diluted 50 times with saline, and the resulting thus dilution (1:50) is considered the original. Next, the initial serum dilution sequentially diluted twice with saline. For this (see diagram productions):

- a) in all agglutination tubes, except for No. 6, 1.0 ml of physiological solution;
- b) 1.0 ml of serum is added to test tubes No. 1 and No. 6 in the initial dilution of 1:50, and, so the way serum in vial no. 1 getting divorced twice more, then eat in one hundred once;
- c) 1.0 ml of serum from tube No. 1 is transferred to test tube No. 2 to those available in her 1.0 ml physiological solution, due to what serum getting divorced more twice then eat in 200 once, and So Further, up to before test tubes No. five, where the dilution reaches 1:1600;
- d) it is obvious that tubes No. 1 - No. 4 contain 1.0 ml of serum, while vial 5 contains 2.0 ml of ee - the excess 1.0 ml is removed, and thus volumes in test tubes No. 1 - No. 5 are equalized. In test tube No. 6, serum control. Further, in each test tube, with the exception of test tube No. 6, add 2 drops DIAGNOSTICUM — processed formalin suspension in physiological cell culture solution salmonella typhi, in each milliliter of which contains 2 billion bacterial bodies. The test tube rack is shaken and placed in a thermostat at t 37°C for 2 hours. After exposure in a thermostat, the reaction rack is kept at room temperature or "in the cold" (+3° +5°C) in for 18 hours.

Components reactions	Exper ience					serum	diagnosticia n crazy
	1	2	3	four	five	6	7
1. Phys. Solution	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2. Researched serum (1:50); ml	1.0 1:100	1.0 1:200	1.0 1:400	1.0 1:800	1.0 1:1600	1.0 1:100	1.0
3. Diagnosticum, drops	2	2	2	2	2	-	2

The results are recorded in a day in the following sequence: first turn evaluate the state of control test tubes (No. 6 and No. 7), second turn

—experienced. AT test tube #6 (control serum) must to be absolutely transparent, devoid of any sediment liquid. AT test tube #7 (control diagnosticum)

- Uniform haze. The results of test tubes should be evaluated starting from test tubes with the highest serum dilution (No. 5). The result of the reaction is taken into account according to fallout on bottom test tubes flakes agglutinate and simultaneous enlightenment content test tubes; at lung tapping on wall test tubes or careful agglutinate is easily separated from the bottom by shaking, floats up and, without changing its structure, returns in initial position.

III. *Reaction ring precipitation*

The precipitation reaction is most often used to determine the presence in the material soluble

antigens. AT control precipitation test tube approximately before

half of its volume is brought in by normal serum. The same amount of precipitating serum. Next, a small amount is added to each tube. the amount of material to be tested - for example, an extract from the skin of an animal (sheep), died presumably from anthrax. The material to be tested should be through cautious layering on internal wall precipitation test tubes, held in the hand at a height of 30-35 cm from the surface of the desktop at an angle of 45 ° to horizontal.

AT experienced test tube on border serum and researched material observed education precipitate: whitish "disk" irreversibly collapsing at shaking test tubes. AT control test tube education precipitate not observed.

IV. Reaction indirect (passive) hemagglutination (RITA)

RPGA is based on the use of erythrocytes with adsorbed on their surface antigens (erythrocyte diagnosticum), the interaction of which with the corresponding antibodies in the blood serum of patients causes erythrocytes to precipitate to the bottom test tubes (wells) in form "revealed umbrella."

The studied serum of the patient is diluted 10 times and heated at 65 ° C for 20 minutes for water bath for removal non-specific hemagglutinins, then cook row her dilutions from 1:100 to 1:3200 and poured into wells of 0.5 ml. Add to each well 0.5 ml diagnosticum. The corresponding erythrocyte is added to each row of wells. diagnosticum: to shigella Sonne, Flexner, Newcastle and polyvalent salmonella.

Simultaneously put controls diagnosticums and control researched serum. The result of the reaction is taken into account after incubation in a thermostat for 2 hours at 37°C or at room temperature for 1824 hours. The reaction is considered positive when condition location erythrocytes in the form of an umbrella all surfaces hole bottom and evaluated like "+".

Scheme productions

Breeding researched serum	DIAGNOSTICS				CONTROL				
	Sonne	Flexner	New castle	Salmon. watered.	cd 1	cd 2	cd 3	cd four	Ks
1:100									
1:200									
1:400									
1:800									
1:1600									
1:3200									
Incubation at t 37 ° C; 24 hours.									
Accounting results									

TIMELINE

- | | | |
|--|-------|-------------|
| 1. Definition original level knowledge | ----- | thirty min. |
| 2. Independent Job | ----- | thirty min. |
| 3. Examination protocols | ----- | 10 min. |
| four. Cleaning working places | ----- | 10 min. |
| five. Control final level of knowledge and task on house | ----- | 10 minutes. |

PRACTICAL OCCUPATION #14

Theme: Serological reactions. Reactions immunity With participation complement.
Reactions immunity With labeled components.

Educational goal:

1. Explore complement dependent serological reactions,
2. Explore reactions immunity With labeled components.

Plan lessons:

7. Reactions immune lysis, Components.
8. Reaction hemolysis.
9. Reaction binding complement (RSK). Staging and accounting reactions binding complement.
10. Reaction immunofluorescence, straight and indirect.
11. ELISA analysis, Components, application.
12. radioimmune analysis, Components, application.

Independent work of students: 1.

Setting and accounting for the complement fixation reaction with the goal serodiagnosis syphilis

EQUIPMENT

1. Setting up a complement fixation reaction with a target serodiagnosis syphilis:
Researched serum-8 pcs.
Cardiolipin antigen - 8 pcs.
Complement 1.0-4 PC.
test tube With physical solution 4.0 - four PC.
Test tube with a suspension of sheep erythrocytes - 8 pcs.
Test tube with hemolytic serum - 8 pcs.
Clean test tubes 2 PC. - 8 sets.
The pipettes are sterile. ThermostC.
2. Demonstration: RSK, ELISA.
3. Demonstration REEF.
4. Demonstration of bacterial preparations: antigen, complement, hemolysin, immunofluorescent serum;
5. Tables.

INFORMATIONAL MATERIAL ON THEME LESSONS:

principled scheme productions reactions binding complement

Components reactions	tube number		
	1(exp)	2 (counter.)	3 (counter.)

1. Исследуемая сыворотка (1:5)	0,5	0,5	-
2. Антиген в рабочей дозе	0,5	-	0,5
3. Комплемент в рабочей дозе	0,5	0,5	0,5
4. Физиологический раствор	-	0,5	0,5
Incubation at t 37 °C - 40 minutes.			
5. Hemolytic system (sheep erythrocytes + hemolytic serum)	1.0	1.0	1.0

Incubation at t 37 °C — 40 minutes.
Accounting results Hemolysis + Hemolysis +

Conclusion:

At availability antibodies in researched serum (positive reaction) in experienced test tube hemolysis absent. At negative reactions (No antibodies) in all three test tubes observed hemolysis.

The complement fixation reaction takes place in two phases: 1st phase - interaction researched serum With antigen and complement. 2nd phase — indicator — determination of the presence of free complement in the mixture by adding hemolytic systems, consisting from erythrocytes ram and hemolytic serum, containing antibodies to erythrocytes ram. If in first phase reactions going on education antigen-antibody complex, complement is bound by this complex in the second phase hemolysis erythrocytes absent (reaction positive). If antigen and antibody not correspond friend friend, complement in the first the reaction phase remains free and during second phase reactions joins to complex erythrocyte-hemolytic serum, causing hemolysis (reaction negative).

TIMELINE

1. Definition original level knowledge	-----	thirty min.
2. Independent Job	-----	thirty min.
3. Examination protocols	-----	10 min.
four. Cleaning working places	-----	10 min.
five. Control final level of knowledge and task on house	-----	10 minutes.

PRACTICAL OCCUPATION #15

Theme: Immune status.

Educational goal:

1. Explore tests first and second level, then clinical interpretation.

Student should know:

1. Age peculiarities immune status.
2. Methods research lymphocytes, assessment functional state phagocytes,

The student must be able to:

1. Decide and take into account the functional condition phagocytes,
2. Define activity complement blood

Plan lessons:

1. Immune status and principles his estimates.
2. Age peculiarities immune status.
3. Methods research lymphocytes, grade functional states phagocytes,
4. Definition complement
6. Tests first and second level, then clinical interpretation.

Independent Job students:

1. staging and accounting functional states phagocytes,
2. Definition complement

EQUIPMENT

1. Immunograms
2. smears With phagocytosis

INFORMATIONAL MATERIAL

Maturation immune reactivity fetus

thymus is being laid on second month intrauterine life in areas third- fourth gill pockets and on sixth week It has expressed epithelial character. At 7-8 weeks, it is "populated" with lymphocyte-like cells. By the end of the third month, the formation of the organ ends. Later in the thymus, there are only quantitative changes. Lymphatic nodes and other secondary organs of the immune system systems are laid on 4th month, then final formation ends in postnatal period. Lymphoid follicles located in the ileum and appendix, in Peyer's patches contain "cells predecessors" plasmatic cells. They are ripen before plasma cells, synthesizing IgA to 14-16 week intrauterine development fetus.

Stem cells appear at 3-8 weeks of embryogenesis and are found in the liver, blood islands of the yolk sac. Later, their main place of education becomes Bone marrow. Lymphocytes are first detected at week 9 in the thymus, at 12-15 spleen. In the blood, lymphocyte-like cells are determined from 8-10 weeks. Lymphoid cells, endowed function T-lymphocytes, come to light on 10-11 week. B cells determined in liver With 10- 12, in spleen — With 12 weeks. Synthesis and secretion IgM registered in cells on 11th IgG- on 22nd week. Content IgM is 1/10 from

maternal, a IgG — more smaller. Education components systems complement starts at fetus on 8th week pregnancy. At this Components C2 and C4 synthesized macrophages, C5 and C4 - in liver, lungs peritoneal cells NW and C1

— in thin and thick gut. On 18th week development Everybody specified Components determined in serum blood fetus. Cellular and humoral factors non-specific anti-infectious immunity appear in early ontogeny.

During the period of embryonic development, the "work" of the immune system has its own characteristics. AT in particular among T-dependent immunological reactions, the ability to rejection transplant (13 a week), HRT implemented much later.

Despite on Availability in body fetus significant quantities B cells With immunoglobulin receptors plasmatic cells, directly synthesizing AT, very few. Row very powerful factors suppresses function humoral part of the immune system. It is a choriotropic gonadotropin, a-fetoprotein, a-2-globulin. Sharp limited in this period influence on B-cell- ki T-lymphocytes and macrophages.

Premature activation immune systems observed at intrauterine infection. Practically always this is accompanied by any immunopathological disorders. So the way for embryonic period a typical step in immunogenesis is the tolerance of one's own immune system and passive antibody immunity per check maternal IgG, concentration which progressively is growing in process pregnancy. Ability fetus form Components systems complement defective. AT III trimester her level although and increases but is not more 30-50% indicators adults. System local immunity in early and late ontogeny not developed.

immune status at children after birth

A healthy full-term baby born to a healthy mother with a physiological course pregnancy, has a certain immune status and an appropriate level of factors non-specific anti-infectious resistance. Peculiar character passive immunity newborn It has positive and negative sides. So, not receiving from mothers IgM, fetus not saturates related With this class group isohemagglutinins, which reduces the risk of conflict in case of mismatch of group erythrocyte Ag. FROM another sides induced low protection vs gram-negative bacteria, since this fraction mainly contains AT vs specified pathogens. AT moment birth at child observed physiological leukocytosis, reaching before 12-15 billion cell/l. From cells more 35% make up lymphocytes. Of the total number of lymphocytes, about half are T cells. In relative terms, their content is moderately reduced, and in absolute terms, given tall leukocytosis, not changed. About 60% all T-lymphocytes constitute cells With helper functions, 15% — T-suppressors.

Content antibody-dependent killers same strongly reduced from level adults. Functions lymphocytes newborns changed. So, intensity reactions blast transformation induced by PHA T-mitogen is "normal" or slightly reduced, how at more senior children. reduced them ability produce lymphocytes,

induce skin reactions. At the same time, the cells show a higher level metabolism, if judge on intensity nucleic synthesis acids.

The number of B cells in newborns is usually increased in relative and absolute values. As rule on these cells are found IgM and IgE receptors. AT cord blood of newborns, IgM and IgG are detected, IgA and IgE are detected extremely seldom. Synthesis IgM sharp increases reaching maximum on 2-3 weeks life child, then by the age of one month it decreases, then slowly increases, reaching by 6-12 adult level. An excessive increase in the concentration of IgM in newborns is evidence of intrauterine infection. Most often it is syphilis, rubella. A three-fold increase in IgM levels is evidence of sepsis in child.

The concentration of IgG is very low at birth, increases by 7-8 years. In children fed artificially, this dynamics implemented much faster. IgA in serum blood newborns, How rule missing in flow first months life. In the future, the content of immunoglobulin slowly increases, reaching by the end the first year 28% of the level of this protein in adults. Normalization of the parameter is achieved

by 8-15 years. IgD at newborns usually not is determined. Appears this protein approximately on 6th week, reaching level adults to 5—10—15 years. IgE same not is found at newborns, gradually growing, he approaching to values adults by the age of 11-12. Acceleration of reagin accumulation is a risk of development in children bronchial asthma and others allergic and especially atopic diseases.

It is known what content immunoglobulins determined sum AT various specificity. Earlier than others in children, the appearance of immune globulins has an effect microflora organism child. Main representative intestinal microflora in this

period are bifidumbacteria. therefore any unfavorable factors (artificial feeding, application antibiotics) inevitably entail per yourself violation specific composition microflora and changes spectrum emerging C. Antibody formation in newborns, as a rule, proceeds only according to the primary type, requiring for the implementation of a large amount of Ag. Significantly slow switchingsynthesis with IgM on IgG, in flow 5-20 days at adults and 20-40 — at children.

At the time of birth, phagocytes and blood serum of newborns have a certain bactericidal activity vs row microbial strains. Chemotaxis and functional activity macrophages reduced. Partially this is compensated increase content granulocytes, So same endowed phagocytic function. However, digesting ability these cells lowered per check enzyme immaturity.

A child is born with reduced complement levels compared to adults. properdin, which grow quite quickly. The initial activity of lysozyme, on the contrary, significant.

Content lysozyme in body not always, depends from age, time of the year, vitamin balance, etc. Most of the lysozyme in the saliva of children (up to 200 mcg / ml), which many times higher than its concentration in blood serum. The highest content lysozyme in the saliva of children of the first year of life, at the age of 1-6 years it decreases by almost 3 times, to 7-15 years increases but not reaches original level. Important factor local immunity is IgA, which is in two forms - serum and secretory. This y-globulin plays a major role in the body's resistance against respiratory, viral, bacterial, parasitic infection, etc. Secretory IgA starts show up in secrets first and early second weeks continues progressively increase in the following months and years, in coprofilters it is found with third week of life. The amount of secretin is constantly replenished due to the secretory IgA of milk and, especially, colostrum, where its amount is 20 times or more higher than the level in adult serum. Usually after 3-5 days of lactation, the concentration of IgA decreases sharply, but, given the increasing consumption of milk by the child, its amount Plasma cells located in the mucous membranes form IgA, IgM, IgG, IgD, IgE. Wall intestines synthesizes before 3 G immunoglobulins in day. IgG provide protection in mostly vs toxins rest vs bacteria and viruses. Formation full-fledged local immunity, according to various sources, ends by one to twelve years life.

Ratio plasmatic cells gastrointestinal track, producing immune globulins, changes in some diseases. Yes, in young children (from birth and up to three years old) With chronic gastroduodenitis there is an IgA deficiency and increase products IgM. At patients With cholecystitis celebrated decrease IgA concentrations and an increase in IgM or IgG. With peptic ulcer of the duodenum 12 going on the fall level IgA in duodenal content. deficit local IgA facilitates binding immune globulins others classes With Ag.

Local immunity conditioned not only humoral, but and cellular factors. Shown what in the first 24 hours after birth baby going sharp increase in the number of alveolar macrophages. Their number continues to increase monthly age, after what stabilizes. Microbicidal properties macrophages and others phagocytic cells, How rule behind at children first weeks and even months life.

State immune systems child in first years life characterized high dynamism. So, after birth declining number leukocytes in circulation, rises percentage content lymphocytes, decreases

number granulocytes.

The crossover between the curves reflecting the dynamics of these cells occurs for the first time at 5 day of life, after which a similar cross (decrease in the proportion of lymphocytes and increase in neutrophils) celebrated only in age 4-5 years old. Highly slowly rises relative content T cells level B-lymphocytes steadily declining before norms.

Thus, for the embryonic period, tolerance and passive immunity per check maternal IgG, concentration which is growing in process pregnancy. In the newborn, maternal passive immunity also dominates, although already celebrated Start synthesis own AT, endowed small 12 months immune reactivity matures. AT age 1-3 years old distinctly working T cell immunity. AT this same period active are functioning and B-lymphocytes.

From the foregoing it follows that the body of a newborn up to one year of age is badly protected from infectious agents. Active main way humoral link immunity. T-dependent reactions and phagocytosis are underdeveloped and enter into full force later. Sometimes only to period of sexual maturation. Considering Everybody these intelligence the appointment of children with immunotropic drugs should be done with extreme caution so that do not distort the natural features of the reaction, mistaking for immune disorders physiological changes immune reactions.

In many diseases in children, the liver and spleen. These bodies in intrauterine period carry out hemo- and lymphopoiesis. therefore in answer on damage or infection fetus answers activation reticuloendothelial system. After birth, its significance decreases, being replaced by more committed mechanisms. However, at parts So called "slowly starting children" With delay maturation immune systems possible reaction on pathogenic situation the indicated bodies. AT the present time in life child allocate some critical periods, which are characterized by the greatest vulnerability of the body (D.V. Stephanie Yu.E. Veltishchev, 1996). In intrauterine period critical should think age 8-12 weeks, when the differentiation of organs and cells of the immune system occurs. The first critical period after birth is the neonatal period, when organism exposed action huge numbers Ag. immune system in this is time exposed strong suppressive influences passive humoral immunity conditioned maternal C. noted functional imbalance T-lymphocytes, suppressor function implement not only CD8+ cells, but and immature thymocytes and other cells.

Second critical age (3-6 months) characterized weakening passive humoral immunity due to the catabolism of maternal C. At the same time, the suppressor the direction of immune peaktia is consistent with the presence of pronounced lymphocytosis. Such type immune response comes at vaccination vs tetanus, diphtheria, whooping cough, poliomyelitis, measles, and only after the 2-3rd revaccination does secondary immune answer With education IgG AT and persistent immune memory.

The third critical period is the 1st year of life. At this time, the primary character is preserved immune response to many antigens, however, it is already possible to switch to the formation of IgG- C. However synthesis subclasses IgG2 and IgG4 is late. suppressor orientation immune mechanisms starts to change to helper. Local immunity is not developed, children sensitive to respiratory viral infections. Fourth the critical period is the 4th-6th years of life. At this age, the average concentration of IgG and IgM in the blood corresponds to that in adults, the concentration of IgA in plasma does not yet reach final values, the content of IgE in the blood reaches its maximum values. The period is characterized by a high frequency of atopic, parasitic, immunocomplex diseases.

The fifth critical period is adolescence (in girls from 12-13, in boys from 14-15 years old). Pubertal jump growth combined With decrease masses lymphoid organs. Raise secretions genital hormones (before Total androgens) leads to suppression cellular link immunity and stimulation his humoral mechanisms. In general, children have the following features of links immune status. T - link of immunity. Peripheral blood lymphocyte count at birth in first day life is 24-30%, and the absolute number — $3-9 \cdot 10^9$ /l. Then their relative number increases and by the 4-5th day reaches 40-50%, absolute — $2.5-10$ billion/l

Lymphocytes newborns different high metabolic activity, in them increased synthesis of DNA and RNA. BTL during cultivation with PHA is well expressed as in full-term, So and at premature newborns. noted tall level spontaneous transformation, on average, about 6-10%, while in adults this figure is about 0.2%. B - link of immunity. The humoral immune system is different from cellular starts actively function only after birth under influence antigenic irritation. At birth child content IgG in his blood usually more than the mother, since the transplacental passage of this immunoglobulin is active process. IgM in serum usually missing or determined in minimal quantities. IgA usually missing or are in trace concentrations. By the end of the first week, the content of IgA and IgM increases slightly, IgG - by the 2nd-3rd week it noticeably decreases and reaches the minimum concentrations at the age of 1-4 months phagocytic unit. The number of neutrophils in the blood at birth is relatively high: 50- 70% and 4.5-20 billion/l. From the 4th day, it begins to decrease to 30-40% - 2.5-6 billion / l. Monocytes during the entire period of the newborn TM make up 4-9% - 0.6-2 billion / l. Absorption ability neutrophils newborns not lowered but digesting activity lowered what leads to unfinished phagocytosis. Number HCT-positive neutrophils in spontaneous reactions at children first 2 weeks life is 14-20%, while in older children it is 2-10%. The rise in the number of these cells in the induced test is low, i.e. phagocytic reserve of cells in children aged two weeks small. Monocytes newborns characterized low bactericidal activity and insufficient migratory ability.

TIMELINE

1. Definition original level knowledge	-----	thirty min.
2. Independent Job	-----	thirty min.
3. Examination protocols	-----	thirty min.
four. Cleaning working places	-----	thirty min.
five. Control final level of knowledge and task on house	-----	10 minutes.

PRACTICAL OCCUPATION #16

Topic:

Immunodeficiencies

Educational goal:

1. Explore pathogenesis secondary immunodeficiencies
2. Explore genetics immunodeficiencies, peculiarities inheritance.
3. Explore congenital immunodeficiencies

Plan lessons

5. Genetics immunodeficiencies, peculiarities inheritance.
6. Congenital immunodeficiencies (classification, diagnostics)
7. Congenital immunodeficiencies at children.
8. Secondary immunological deficiency (SID) - classification, etiology,diagnostics

Independent Job students:

1. Assess and interpret indicators of the immune status in secondaryimmunological insufficiency on ready immunograms

EQUIPMENT

1. Ready immunograms
2. Situational tasks
3. Tables

INFORMATIONAL MATERIAL

Immunodeficiencies (IDS) — violations immunological reactivity, caused by loss of one or more components of the immune apparatus or closely interacting With him non-specific factors.

There is no single classification. By origin, immunodeficiencies are divided into primary and secondary. Contents [put away]

1 Primary immunodeficiencies

1.1 Definition and classification

1.2 Clinical painting IDS

1.3 Treatment primary IDS

2 Secondary immunodeficiencies

2.1 Causes

2.2 Treatment of secondary IDS

Definition and classification

Primary immunodeficiencies - this is congenital (genetic) or embryopathies) defects immune systems. AT depending on level violations and localization defect they there are:

humoral or antibody — With predominant defeat systems AT- lymphocytes)

X-linked agammaglobulinemia (Bruton's disease) Hyper-

IgM syndrome

X-linked autosomal

recessive

deletion of immunoglobulin heavy chain genes

deficit k-chains

selective IgG subclass deficiency with or without IgA deficiency

deficiency of antibodies with normal levels of immunoglobulins

general variable immune failure

IgA

deficiency

cellular

Dee syndrome Georgie primary

deficiency of CD4 cells deficit

CD7 T cells

IL-2 deficiency

multiple cytokine deficiency defect

transmission signal combined:

syndrome Wiskott-Aldrich

ataxia-telangiectasia (syndrome Louis Bar)

severe combined immunodeficiency X-linked With floor

autosomal recessive deficit

adenosine deaminase

deficit purine nucleoside phosphorylase

deficit molecules II class WPC (syndrome bald lymphocytes) reticular dysgenesis

CD3 γ or CD3 ϵ deficiency

deficit CD8 lymphocytes

insufficiency of the complement system

defects phagocytosis

hereditary neutropenia

infantile lethal agranulocytosis (Kostman's disease) cyclic

neutropenia
 familial benign neutropenia defects in
 phagocytic function chronic granulomatous
 disease
 X-linked autosomal
 recessive
 type I lymphocyte adhesion deficiency
 deficit adhesion leukocytes 2 type
 neutrophil glucose-6-dehydrogenase deficiency
 deficit myeloperoxidase
 deficit secondary granules

Shwachman's syndrome Clinical
 picture of IDS Clinic It has row
 general heck:

1. Recurrent and chronic infections top respiratory ways, paranasal sinuses, skin, mucous membranes, gastrointestinal tract, often called opportunistic bacteria protozoa, mushrooms, having trend towards generalization, septicemia and torpid to ordinary therapy.
2. Hematological deficits: leukocytopenia, thrombocytopenia, anemia (hemolytic and megaloblastic).
3. Autoimmune disorders: SLE-like syndrome, arthritis, scleroderma, chronic active hepatitis, thyroiditis.
4. Often, IDS is combined with type 1 allergic reactions in the form of eczema, edema Quincke, allergic reactions on introduction medicinal drugs, immunoglobulin, blood.
5. Tumors and lymphoproliferative diseases in IDS occur 1000 times more often, how without IDS. [1]
6. At sick With IDS often are celebrated disorders digestion, diarrheal syndrome and syndrome malabsorption.
7. Patients with IDS are characterized by unusual reactions to vaccination, and the use of at them alive vaccines dangerous development sepsis.
8. Primary IDS often fit together With vices development, before Total With hypoplasia cellular elements cartilage and hair. Cardiovascular vices described, main the way at syndrome Dee George.

[edit]

Treatment primary IDS

Etiotropic therapy is in corrections genetic defect methods genetic engineering. But this approach is experimental. Main efforts at established primary IDS directed on:

prevention infections

substitution correction defective link immune systems in form transplants bone brain, substitution immunoglobulins, transfusions neutrophils.

enzyme replacement therapy cytokine

therapy vitamin therapy

treatment of associated infections

Secondary immunodeficiencies

Factors capable call secondary immunodeficiency, very varied. Secondary immunodeficiency can be caused by both environmental factors and internal factors organism. AT in general, Everybody unfavorable factors environmental environment, capable break exchange substances organism, may become cause development secondary immunodeficiency. To most widespread environmental factors that cause immunodeficiency include pollution environmental environment, ionizing and microwave radiation, sharp and chronic poisoning, long-term use of certain drugs, chronic stress and overwork. General trait described higher factors is complex negative impact on all body systems, including immune system. Except Togo, such factors How ionizing radiation render electoral inhibitory action on immunity related With oppression systems hematopoiesis. People, living or working in

conditions polluted environmental environment, more often get sick various infectious diseases and more often suffer oncological diseases.

Obviously, what such promotion incidence at this categories people related codecline activity immune systems.

Causes

Secondary immunodeficiencies are a common complication of many diseases and states.

Main causes secondary IDS:

defect nutrition and general exhaustion organism same leads to decrease immunity. On background general exhaustion organism violated Job all internal organs. immune system especially sensitive to lack vitamins, minerals and nutritional substances So How implementation immune protection is an energy-intensive process. Often a decrease in immunity is observed during seasonal vitamin insufficiency (winter sprind)

chronic bacterial and viral infection, a same parasitic invasions(tuberculosis, staphylococcosis, pneumococcosis, herpes, chronic viral hepatitis, rubella, HIV malaria, toxoplasmosis, leishmaniasis, ascariasis and etc.). At various chronic diseases infectious character immune system undergoes serious changes: violated immunoreactivity, develops increased sensitization on relation to various antigens microbes. Except Togo, on background chronic infectious process observed intoxication organism and oppression functions hematopoiesis. Immunodeficiency during _ infections HIV

mediated by selective damage to cells of the immune system by a virus helminthiasis loss of immune defense factors is observed during severe blood loss, with burns or kidney disease (proteinuria, chronic renal failure). The common feature of these pathologies is significant loss plasma blood or dissolved in her proteins, part of which is immunoglobulins and others components immune systems (proteins systems compliment C-jet protein). In time bleeding is lost not only plasma, but and cells blood, so on background strong bleeding decline immunity It has combined character (cellular humoral)

diarrhea syndrome

stress syndrome

heavy injury and operations same flow co decline functions immune systems. In general, any serious disease of the body leads to a secondary immunodeficiency. Partly this is related With violation exchange substances and intoxication organism, a partly With topics what in time injuries or operations stand out large quantities hormones adrenal, which oppress function immune systems

endocrinopathy (DM, hypothyroidism, hyperthyroidism) lead to a decrease in immunity for check violations exchange substances organism. Most expressed decline immune reactivity of the organism is observed in diabetes mellitus and hypothyroidism. At these diseases declining production energy in fabrics, what leads to violation processes division and differentiation cells, in volume including and cells immune system. Against the background of diabetes mellitus, the frequency of various infectious diseases are on the rise. This is due not only to the inhibition of the function immune systems, but and With topics what elevated content glucose in blood sick diabetes stimulates bacterial growth

sharp and chronic poisoning various xenobiotics (chemical toxic substances, medicines, narcotic drugs). Especially expressed decline immune protection in time reception cytostatics, glucocorticoid hormones antimetabolites, antibiotics

low weight body at birth

decline immune protection at people senile age, pregnant women women and

children related with age and physiological features organism these categories people malignant neoplasms - disrupt the activity of all body systems. The most pronounced decrease in immunity is observed in the case of malignant blood diseases (leukemia) and when red bone marrow is replaced by metastases tumors. Against the background of leukemia, the number of immune cells in the blood sometimes increases tens, hundreds and thousands of times, however, these cells are non-functional and therefore cannot ensure normal immune protection organism

Autoimmune diseases occur due to dysfunction of the immune system. On background diseases this type and at them treatment immune system working not enough and, sometimes not right, what leads to damage own fabrics and inability overcome infection

Treatment secondary IDS

The mechanisms of immune suppression in secondary IDS are different, and, as a rule, there is a combination of several mechanisms, disorders of the immune system are expressed to a lesser extent than in the primary. As a rule, secondary immunodeficiencies are transitory. In this regard, the treatment of secondary immunodeficiencies much simpler and more efficient on comparison With treatment primary violations immune system functions. Treatment for secondary immunodeficiency is usually started With definitions and eliminate causes his occurrence. For example, treatment immunodeficiency on background chronic infections start With sanitation foci chronic inflammation. Immunodeficiency on background vitamin and mineral deficiencies begin to be treated with the help of complexes of vitamins and minerals. Recovery capabilities immune systems are great so elimination causes immunodeficiency, How rule leads to restoration immune systems. For acceleration recovery and stimulation immunity carry out well treatment with immunostimulating drugs. At present, it is known number immunostimulating drugs, With various mechanisms of action.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #17

Change module: "Infectious immunology. Reactions immunity. Immune status. Immunodeficiency"

№ ЛД-21

**FEDERAL STATE BUDGETARY EDUCATIONAL INSTITUTION HIGHER EDUCATION
"NORTH OSSETIAN STATE MEDICAL ACADEMY»
MINISTRY OF HEALTH RUSSIAN FEDERATION**

DEPARTMENT MICROBIOLOGY

**COLLECTION METHODOLOGICAL DEVELOPMENT
ON MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY FOR
TEACHER FOR MEDICAL FACULTY**

AUTUMN SEMESTER

Vladikavkaz

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Main appointment developments - methodical help teachers to each practical occupation in autumn semester. Directions drawn up in accordance with the Federal State Educational Standard of the Higher and professional education.

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PRACTICAL OCCUPATION No. 1.

TOPIC: STAPHYLOCOCCI. MICROBIOLOGICAL DIAGNOSIS staphylococcal DISEASES.

TRAINING GOAL:

1. Explore biological properties staphylococci.
2. Explore methods microbiological diagnostics staphylococcal diseases.

PLAN LESSONS:

1. Morphology, cultural and biochemical properties staphylococcus.
2. Factors virulence staphylococcus.
3. Antigens staphylococcus.
4. diseases, called staphylococcus.
5. Diagnostic methods and test material in staphylococcal diseases.
6. Preparations for specific prevention and treatment
7. staphylococcal diseases.

INDEPENDENT JOB STUDENTS:

1. Explore morphology staphylococcus in smear from clean culture, describe sketch.
2. To give macroscopic characteristic colonies on milk-salt agar (bacteriological diagnostic method, 1st stage research).
3. To identify culture staphylococcus on morphological, cultural, biochemical properties, define virulence factors (2nd stage bacteriological method):
 - a) recording the results of inoculation of staphylococcus culture on blood agar with a goal definitions hemolysin.
 - b) accounting results sowing in citrate plasma for definitions plasmacoagulase.
 - c) accounting seeding results on yolk-salt agar for the purpose definitions lecithinases.
 - d) accounting seeding results on Wednesday With mannitol.
4. describe drugs for specific therapy and prevention staphylococcal diseases (staphylococcal toxoid, antistaphylococcal plasma, antistaphylococcal immunoglobulin, staphylococcal bacteriophage).
5. Decor protocol research.

EQUIPMENT

1. Set for bacteriological research Tripod - 8 pcs
Tweezers - 8 PC. Bacteriological
loop - 8 pcs. Bottle With physical
solution - 8 pcs
Spirit lamp - 8 PC.
2. Microscopes - 8 pcs.
Immersion butter
3. Paint set on Gramu – 8 pcs.
4. cups With Kas growth of staphylococcus aureus - 8 pcs
5. Tubes with MPA With growth of staphylococcus 8 pcs
6. test tubes with BCH - 8 PC.

7. cups petri With lecithinase activity- 8 PC
8. Petri dishes With milk-salt agar and the growth of staphylococcus-8pcs
9. Tubes with curled up citrate plasma - four PC.
10. Bacterial preparations.

METHODOLOGICAL RECOMMENDATIONS

The main method for diagnosing staphylococcal diseases - bacteriological. For allocation clean culture researched material sow on yolk-salt, blood or milk-salt agar. Grown isolated colonies are subcultured on oblique agar for receiving clean culture.

Identification clean culture carry out on morphological, cultural, biochemical properties, then define factors virulence.

I. DEFINITION HEMOLYTIC ACTIVITIESBACTERIA.

A culture of staphylococcus was made on a blood agar plate. The cups are left in thermostat on 24 hours at temperature 37 degrees.

When evaluating the results, attention is paid to areas of hemolysis, i.e. enlightenment of the environment around grown colonies. Hemolytic properties bacteria connected With presence hemolysin (exotoxin).

IV. DEFINITION LECITINASES

Staphylococcus aureus was inoculated on a plate with yolk-salt agar. The cups are left in thermostat on 24 hours.

When evaluating the results, the presence of turbidity haloes around the colonies is taken into account, which testifies about education staphylococcus aureus lecithinase enzyme.

V. FOR DETECTIONS ENZYME PLASMACOAGULASES

Produce sowing culture staphylococcus in citrate plasma. test tubes put in thermostC. The results are taken into account after 24 hours. In the presence of the enzyme plasmacoagulase going on coagulation plasma With education clot fibrin. Availability enzyme plasmacoagulase is the main identification feature of the species S.aureus, which often is pathogen nosocomial infections.

IV.DETERMINATION OF MANNITE FERMENTATIONAT ANAEROBIC CONDITIONS

To determine this feature, confirming the belonging of a pure culture staphylococcus to the most aggressive species S.aureus, sowing was done on a medium with mannitol. At cleavage of mannitol, acidic products are formed, which change the color of the indicator in environment (indicator Andrede - gives red coloring environment, a indicator VR - blue).

№№ P/P	researched material	results research	Graphic image

Informational material to topic

Of the 14 species of staphylococci that live on the skin and mucous membranes of humans, prevail and often cause diseases: *S.aureus*, *S.epidermidis*, *S.saprophyticus*. Staphylococcus-gram-positive cocci, motionless, dispute and capsules not form, insmears are arranged in clusters in the form of "clusters of grapes". **Cultural properties** . Not demanding on nutrient media: cultivated on MPA with the formation of pigmented colonies yellow or white colors, in the BCH give

diffusely turbid growth. For the identification of staphylococci, the nature of growth is important on blood agar (hemolysis zone) and yolk-salt agar (YSA) (determination lecithinase).

Biochemical properties . Staphylococci break down carbohydrates into acids. Important a differentiating feature of various types of staphylococci is the formation acids from mannitol in aerobic and anaerobic conditions.

pathogenicity factors .1.

Factors adhesion:

-teichoaceae acids provide adhesion on cells organism;

"hospital strains" *S.epidermidis* produce a special kind of mucus that provides their attachment to polymeric materials of catheters, artificial heart valves and creation of a bacterial biofilm on them. This leads to the development of sepsis and endocarditis, conditioned " hospital strains" *S. epidermidis*.

2. Protein A non-specific binds Fc fragment IqQ that leads to oppression phagocytosis, functions complement and opsonizing actions antibodies.

3. Eclipse antigens, having antigenic commonality With cells skin and kidney person.

8. Enzymes pathogenicity:

– hyaluronidase, splits hyaluronic acid in composition connective fabrics, what promotes the spread of staphylococci;

-plasma coagulase causes clotting proteins serum blood, forming fibrin

"pseudocapsule" that protects staphylococci from phagocytosis

-Plasmocoagulase is one of the important markers of various types of staphylococci for differentiation. *S.aureus* has plasmacoagulase and is coagulase-positive staphylococcus; *S. epidermidis* and *S. Saprophyticus* do not have plasmacoagulase and are classified as coagulase-negative (KOS).

-fibrinolysin splits fibrin and promotes splitting staphylococci in body;

-lecithinase destroys lipid membranes cells organism;

nucleases (RNases, DNases) cleave DNA molecules and RNA, which leads to destruction synthesis squirrel in cells and their death;

- β -lactamase destroys - β -lactam antibiotics (penicillins, cephalosporins).5. **Exotoxins:**

-hemolysins 4th types, in mostly possessing hemolytic and cytotoxic action;

-leukocidin destroys leukocytes;

exfoliatins cause damage and detachment epidermis With accumulation liquids and formation of blisters, causing the development of the syndrome of "scalded skin" (syndrome Lyell);

-toxic shock exotoxin (ECT) causes systemic damage to the body in the form of syndrome toxic shock (STSH) With high lethality;

Enterotoxins cause symptoms of acute food poisoning. All toxins except hemolysins, produces only *S. aureus*.

6. **R-plasmids** (factors multiple medicinal sustainability).

S.aureus - ubiquitous, are part of the facultative microflora of the skin and mucous shells nose and nasopharynx.

Sources infections are sick human and bacteriocarrier. Often formed carriage at medical staff.

Ways of infection: airborne, contact, alimentary. In individuals with reduced resistance available endogenous way infections.

Nosological forms of infections caused by *S.aureus* are diverse, because are amazedany fabrics and organs.

S.epidermidis colonizes the skin and mucous membranes. Most often causes nosocomial, iatrogenic infections: sepsis, endocarditis, urological infectionwhat is associated with the colonization of artificial heart valves by these microorganisms, catheters prostheses vessels.

S. saprophyticus colonizes mucous membranes of the urogenital tract and causes inflammation various departments urinary ways at people With reduced resistance.

Main nosological forms staphylococcal infections

Forms diseases	Material for research
Local	
Purulent defeat skin (boils, carbuncles, abscesses.Phlegmon)	Purulent detachable, purulent content
mastitis	breast milk, pus from abscess
Angina, tonsillitis	Smear from pharynx, With tonsils
Pneumonia, bronchopneumonia	Sputum, flushing water bronchi, blood
Arthritis	articular liquid
Conjunctiva	Purulent detachable conjunctiva
infections urinary ways	Urine
food poisoning	Flushing water stomach, emetic masses, faeces, leftovers food
Generalized	
Sepsis	
Endocarditis	
Meningitis	
Hematogenous osteomyelitis	
Syndrome toxic shock (STSH)	Detachable from vagina, blood

specific treatment staphylococcal infections

Acute staphylococcal infections	Chronic staphylococcal infections
Immunoglobulin staphylococcal human	Anatoxin staphylococcal purified liquid
Staphylococcal bacteriophage	killed staphylococcal vaccine, chemical staphylococcal vaccines forbasis protective antigens

Streptococci are Gram-positive cocci, non-motile, do not form spores or capsules.smears are located chains.

Cultural properties . streptococci demanding to nutritious Wednesdays. AT sugarbroth give near-wall type of growth. On blood agar they form small convex colonies. Optional anaerobes.

By character growth on blood agar allocate 3 groups streptococci:

- 4) α -hemolytic cells form a green zone around the colonies (“greenstreptococci) in result transformation hemoglobin in methemoglobin;
- 5) β -hemolytic cause full lysis erythrocytes and form around coloniestransparent zone;
- 6) γ -streptococci do not cause hemolysis and are non-homolytic. **Biochemical properties** .

When identifying streptococci, their ability to ferment carbohydrates, grow on media with bile, as well as on environments with high concentration NaCl and reduce in milk methylene blue.

Antigenic structure . By antigenic structure (polysaccharide antigens of cell walls) R. Lensfield divided streptococci on 20 serogroups - AND, AT, FROM, and etc. To streptococcus group A include - S.pyogenes (β -hemolytic - streptococcus), the most pathogenic view.

α -hemolytic streptococci are mostly part of the normal microflora ("oral streptococci", enterococci), but can cause pathology in humans when decline residency organism.

Non-hemolytic streptococci are included in composition obligate microflora mucous shells human and usually don't cause pathological processes.

The most epidemiologically significant for humans is the species S.pyogenes, which has significant set **factors pathogenicity**:

4. Factors adhesion : lipoteichoic acid cellular walls;
5. Protein M provides not only adhesion but and suppression phagocytosis;
6. Eclipse antigens having antigenic commonality with cloth hearts and kidneys.

Enzymes pathogenicity:

- hyaluronidase - promotes displacement microbes by connecting fabrics;

-fibrinolysin (streptokinase)- causes dissolution fibrin blood clots, promotes dissemination on circulatory channel;

-DNA-aza- destroys molecules DNA.

Exotoxins :

-hemolysins (O- and S-streptolysins) - have hemolytic and cytotoxic action on cardiomyocytes and phagocytes;

-erythrogenic (pyrogenic) - lead to the formation of rashes on the skin, have pyrogenic action, cause development of the syndrome toxic shock.

A source infection : sick human and bacteriocarrier.

Ways of infection: airborne, contact, for S aqalactiae - intranatal (in time childbirth).

The main method of microbiological diagnosis of streptococcal infections is bacteriological.

PROTOCOL RESEARCH

Stage (day research)	move research	Result
	Microscopy swabs from pus, painted according to Gram	Among leukocytes visible Gr + cocci located

1st	Sowing in cups with bile-salt agar	small bunches and same on alone and in pairs. colony growth medium size with turbidity around colonies and iridescent whisk
2 - th	smear microscopy from selected colonies, painted on Gramu Screening of colonies with rainbowwhisk on oblique agar	AT field vision visible Gr + cocci locatedforms
3rd	Dedicatedclean culture. Feature definition pathogenicity: a) smear microscopy, painted on Gram; b) inoculation on Hiss media withmannitol and glucose anaerobic and anaerobic conditions; c) definition hyaluronidase activity,plasma coagulation, DNase;d) determination of α -hemolysinon blood cups agar; e) phage typing. Sensitivity testto antibiotics method paper disks.	
4th	Conclusionresearch	Highlighted culture pathogenic staphylococcus. Fagotype is --- sensitive to next antibiotics

Scarlet fever is an acute infectious disease, manifested by a small punctate rash, fever, general intoxication, tonsillitis. The causative agent of the disease is group A streptococcus (*Streptococcus pyogenes*). Infection occurs from patients by airborne droplets (withcough, sneezing, conversation), a same through items everyday life (dishes, toys, underwear). Especially dangerous sick like sources infections in first days illness.

Sources pathogen infections are sick scarlet fever or any another clinical form of streptococcal infection and a bacteriocarrier. Get sick more often children aged 3-10 attending kindergartens and schools. The occurrence of cases scarlet fever in children's institutions, How rule preceded by elevated level incidence of tonsillitis and acute respiratory viral infections. Children of the firstyears of life (especially the first half of the year) and adults rarely get sick with scarlet fever. Basic way transmission pathogen infections — airborne.

Pathogenesis

Pathogen penetrates in organism human through mucous shells pharynx and nasopharynx, in rare cases Maybe infection through mucous shells genital bodies or damaged skin. AT place adhesion bacteria formed local inflammatory-necrotic hearth. Development infectious-toxic syndrome conditioned in first turn admission in blood flow erythrogenic toxin streptococci

(toxin Dick), a same action peptidoglycan cellular walls. Toxinemia leads to a generalized expansion of small vessels in all organs, including volume including in skin covers and mucous shells, and appearance characteristic rash. Synthesis and accumulation antitoxic antibodies in dynamics infectious process, binding them toxins in subsequent condition decrease and liquidation manifestations toxicosis and gradual disappearance rash. Simultaneously develop moderate phenomena perivascular infiltration and edema dermis. Epidermis impregnated exudate, his cells exposed keratinization, what in further leads to peeling of the skin after the extinction of the scarlatinal rash. Maintaining a strong connections between keratinized cells in the thick layers of the epidermis on the palms and soles explains large-lamellar character peeling in these places.

Components cellular walls streptococcus (group A-polysaccharide, peptidoglycan, protein M) and extracellular products (streptolysins, hyaluronidase, DNA-aza and etc.) condition development reactions hypersensitivity delayed type, autoimmune reactions, formation and fixation immune complexes, violations systems hemostasis. In many cases them can think cause development glomerulonephritis, arteritis, endocarditis and other complications of immunopathological character.

From lymphatic formations mucous shells oropharynx pathogens on lymphatic vessels enter the regional lymph nodes, where they accumulation, accompanied by development inflammatory reactions with foci of necrosis and leukocyte infiltration. Subsequent bacteremia in some cases maybe lead to the penetration of microorganisms into various organs and systems, the formation purulent-necrotic processes in them (purulent lymphadenitis, otitis, bone lesions fabrics temporal areas, solid cerebral shells, temporal sinuses and etc.).

Scarlet fever should be distinguished from measles, rubella, pseudotuberculosis, medicinal dermatitis. In rare cases, the development of fibrinous deposits, and especially when they go beyond limits tonsils disease is necessary differentiate from diphtheria.

Scarlet fever distinguish bright spilled hyperemia oropharynx ("flaming yawn"), sharp limited in place transition mucous shells on solid sky, bright red language Withraspberry hue and hypertrophied papillae ("raspberry tongue"), punctate elements rashes on general hyperemic background, thickening rashes in form dark red stripes on skin folds in places natural folds, distinctly expressed white dermographism, pale nasolabial triangle (symptom Filatov). At pressure on skin palm rash in this place temporarily disappears ("symptom palms"), endothelial symptoms are positive. After the disappearance of the exanthema finely scaly peeling of the skin is noted (large-lamellar on the palms and soles). *Laboratory diagnostics*

Diagnosis scarlet fever based on clinical (acute Start diseases, fever, intoxication, acute catarrhal or catarrhal-purulent (with septic form disease - necrotic), tonsillitis, abundant point rash, thickening in natural folds skin and laboratory (neutrophilic leukocytosis, increased ESR, abundant height beta-hemolytic streptococci at sowing material from hearth infection on blood agar, increase in antibody titers to streptococcal antigens - M- protein A-polysaccharide streptolysin-O and others) data.

TIMELINE

1. Definition original level knowledge	-----	thirty min.
2. Independent Job	-----	thirty min.
3. Examination protocols	-----	10 min.
four. Cleaning working places	-----	10 min.
five. Control final level of knowledge and task on house	-----	10 minutes.

PRACTICAL OCCUPATION No. 2.

TOPIC: PATHOGENIC DIPLOCOCCIS. MICROBIOLOGICAL DIAGNOSTICS DISEASES, CALLED PATHOGENIC streptococcus and neisseria.CHANGE

MODULE ON THIS TOPIC: " PATHOGENIC COCKIES "

TRAINING GOAL:

1. To study the morphological and cultural properties of pathogenicgram-positive and Gram-negative strepto- and diplococci (Neisserium).
2. master main methods laboratory diagnostics diseases, calledpathogenic diplococci.

PLAN LESSONS

1. Morphological characteristic pneumococcus (Streptococcus pneumoniae), meningococcus, gonococcus.
2. Comparative characteristics of biochemical activity and the need fornutritional environments for diplococci different types.
3. Differential diagnostic signs (differences) of pathogenic and non-pathogenicneisseria.
4. Factors virulence pathogenic diplococci.
5. A source infection, way transmission, input gates at diseases, causeddiplococci.
6. researched material and main methods diagnostics at pathologicalprocesses, called diplococci.

INDEPENDENT JOB STUDENTS

1. Studying morphology pneumococci (str. pneumoniae) in smears- prints from bodies white mice, infected intraperitoneal sputum sick pneumonia. coloring by Gramu (table).
2. The study of the biochemical activity of pneumococci in order to differentiate them from streptococci. Sowing on environments With inulin and bile.
3. Microscopic method for diagnosing acute gonorrhea: purulent smear microscopy discharge from the urethra sick acute gonorrhea. Coloring with methylene blue.
4. Serological method diagnostics chronic gonorrhea: estimate demonstration reaction complement fixation (by Borde-Jangu), delivered With goal detection antibodies in patient's serum gonorrhea.
5. Decor protocol research.

EQUIPMENT

- 6) Kit for bacteriological research

Tripod - 8 pcs.
 Tweezers -8
 PC.
 Bacteriological loop -8 pcs.Bottle
 With physical solution - 8pcs
 Spirit lamp -8 PC.
 7) Microscopes - 8 pcs.
 Immersion butter
 8) Paint set on Gramu – 8 pcs.
 9) smears With separable urethra sick gonorrhea
 10) RSK (demonstration)
 11) Tank drugs

METHODOLOGICAL RECOMMENDATIONS

5. At contagion white mice sputum sick pneumonia mouse dies from pneumococcal sepsis. Imprint smears are prepared from the organs of a dead mouse. Paint by Gram. On pink background, educated cells fabrics, are found Gram-positive diplococci, slightly elongated, resembling the contours of a flame candles or lancet, surrounded colorless capsule.

6. characteristic sign pneumococcus, distinguishing them from majority others species of streptococci, is related to bile and bile salts. Bile is not only kills, but and dissolves pneumococci. Against, in difference from verdant (*S.faecalis*, *S.sanguis*) and hemolytic streptococci (*S.pyogenes*), pneumococci decompose inulin.

7. Acute gonorrhea is diagnosed by microscopic examination. From researched material do two strokes, one color on Gramu, another - methylene blue. At availability in smear gonococci visible gram negative diplococci, located inside leukocytes (unfinished phagocytosis).

8. Since in chronic gonorrhea gonococci are outside the cells, they have an atypical shape in the form of balls or very small formations, use bacterioscopic method for productions diagnosis is not possible. Therefore, for the diagnosis of chronic gonorrhea apply: bacteriological, serological methods research.

Serological diagnosis gonorrhea put With help RSK. Reaction put for detection antibodies in serum blood sick, With help famous antigen, which is yourself suspension killed gonococci.

Scheme productions RSK

Components reactions	1st (experience)	2nd (control AD)	3rd (control C)
1. Researched serum (1:5)	0.5	-	0.5
2. Antigen in working dose	0.5	0.5	-
3. Complement in working dose	0.5	0.5	0.5
four. Physiological solution	-	0.5	0.5

on 45 minutes in thermostat

five. Hemolytic system	1.0	1.0	1.0
------------------------	-----	-----	-----

on 45 minutes in thermostat

Accounting results		hemolysis	hemolysis
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Accounting result reactions start With control tubes. At availability hemolysis incontrol test tubes about results reactions judge but experienced test tube.

INFORMATIONAL MATERIAL To TOPIC:

Meningococci (Neisseria meningitides) are Gram-negative, bean-shaped diplococci flagella and dispute not have, in body form capsule.

cultural properties . Very demanding on cultivation conditions. grow on solid and liquid nutrient media containing 20-25% serum (serum agar, whey broth). On a dense medium they form small smooth transparent colonies. Strict temperature optimum - 37 ° C (at other temperatures, meningococci perish) must be created both during cultivation and during transportation material from sick in laboratory.

Among the representatives of the genus Neisseria there are opportunistic species, inhabitants of mucous membranes of the nasopharynx - N. Sicca, N.mucosa, etc. In people with weakened resistance, they may cause diseases clinically similar with meningococcal infection.

Antigenic structure. N meningitides has generic antigens common to all species. Inside the View on capsular polysaccharide antigens distinguish serogroups N meningitides- A,B,C,D,YZ and others

epidemiological outbreaks more often cause pathogens serogroups A,B,C.

Factors pathogenicity of meningococci :

1. Pili - provide adhesion on the cells of the cylindrical epithelium of the nasopharynx. 2.

Ig A-proteases - cleave SIg A molecules, thereby reducing local protection mucous membranes of the nasopharynx;

3. Capsule - protects from phagocytosis;

4. Enzymes pathogenicity : hyaluronidase, neurominidase and others

5. Endotoxin (cell wall LPS) - causes damage to blood vessels, which appears hemorrhages in internal bodies and hemorrhagic rash on skin.

The source of infection is a sick person, or a bacteriocarrier. More often (in 70-80% cases) get sick children first three years old life.

Ways of infection - airborne. Entrance gate of infection - mucous membrane nasopharynx.

Meningococcal infection may occur in several clinical forms, who share on localized and generalized.

Main clinical forms meningococcal infections and material formicrobiological research

FORMS	DISEASES	MATERIAL FOR RESEARCH
Primary localized	meningococcal carriage	smear from nasopharynx
	Spicy nasopharyngitis	
Hematogenous generalized	Meningococcemia	Smear from nasopharynx, blood
	Epidemiological cerebrospinal meningitis, meningoenzephalitis	Nasopharyngeal swab, blood. liquor

Microbiological diagnostics meningococcal infections.

3. Bacteriological method (main) - isolation of a pure culture of the pathogen on serum environments and defining its antibiotic sensitivity.

4. Bacteriological method - uses as a mandatory indicative. AT smears from native material with Gram stain, intracellular the location of bacteria and the characteristic pattern of incomplete phagocytosis meningococci.

Specific prevention of meningococcal infection is carried out only according to epidemiological testimony meningococcal polysaccharide vaccine serogroups AND and FROM.

Gonococci - (*N. Gonorrhoeae*) - gram-negative bean-shaped diplococci, form capsule in body, flagella and dispute Dont Have.

Cultural properties . Demanding to nutritious Wednesdays and temperature optimum - 37 °C. Require freshly prepared moist nutrient media supplemented with native proteins blood, serum, or ascitic fluid. Does not cause hemolysis on media containing blood, on media containing with the addition of milk, gelatin and potatoes not are growing.

For gonococci characteristic pronounced antigenic variability even in within one strain.

Biochemical properties: decompose only glucose with the formation of acid. Proteolytic activity absent, ammonia, hydrogen sulfide and indole not form.

Factors pathogenicity gonococcos :

7. Pili - provide adhesion to the cells of the cylindrical epithelium of the genitourinary ways;
8. Capsule - in freshly isolated cultures has antiphagocytic action;
9. Cellular wall contains endotoxin.
10. Surface protein 1 classes causes to bactericidal factors;
11. Surface protein of class 2 forms a separate protein fraction called turbidity proteins or Ora proteins (turbidity). They are considered the first virulence factors of gonococci, and they cause attachment to epithelium.
12. R- plasmids multidrug resistance factors. For diagnostics apply:

Bacteriological method (main) - isolation of a pure culture of the pathogen on serum media and determination of its antibiotic sensitivity. coloring by Gramu and characteristic picture unfinished phagocytosis gonococci.

The serological method is used for chronic gonorrhea, in the absence of the patient's discharge. Carry out RSC on Borde-Jangu according to the standard scheme, which is positive from 3-4 weeks. Used as an antigen for RSK gonovaccine or antigen from killed gonococci.

Genetic method - determination of sections of the gonococcus genome in the material from a patient with help PCR.

For **specific treatment** chronic forms gonorrhea use killed gonococcal vaccine.

Pneumococci - *Streptococcus pneumoniae* Gram-positive diplococcus, usually lanceolate or arranged in chains, having a polysaccharide capsule, which allows easy "type" them specific antisera. pneumococci motionless, do not form a dispute; facultative anaerobes. When cultivating on artificial nutrient media lose the capsule, move from S - to R-shape. Good grow on blood and serum media. When grown on agar with ram's blood, they form colonies with zone α partial hemolysis and greening of the medium, β complete hemolysis, γ -hemolysis visually invisible hemolysis.

Enzymatic activity of glucose with the formation of lactic acid. Pneumococcus not contains group antigen serologically heterogeneous on AG capsule polysaccharides allocate 84 serovar.

In case of pneumococcal infection, in order to isolate a pure culture of the pathogen, they put bioassay - intraperitoneally infect white mice material from sick.

TIMELINE

1. Definition original level knowledge ----- 30 min.

2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION No. 3

THEME: BACTERIA - PATHOGENS INTESTINAL INFECTIONS
(pathogens intestinal escherichiosis, intestinal yersiniosis).

Learning goal: to train students in the methods of microbiological diagnostics and specific prevention of intestinal diseases.

PLAN:

1. Taxonomy and main biological properties of pathogens of intestinal escherichiosis and intestinal yersiniosis.
2. Epidemiology, pathogenesis, immunity caused diseases. 3. Principles of microbiological diagnostics of intestinal escherichiosis and intestinal yersiniosis.
4. Preparations for etiotropic therapy and specific prophylaxis of intestinal escherichiosis and intestinal yersiniosis.

INDEPENDENT JOB

Bacteriological method research

Selection of clean culture from researched material (excreta of patient).

1. Sowing of researched material on differential-diagnostic Endo (demonstration).
2. Accounting for the results of inoculation of the test material on the Endo medium. Selection of "suspicious" colonies and their studying on environment endo, macroscopic colony characterization (demonstration).
3. Seeding "suspicious colonies" on Ressel's medium and BCH.
4. Registration of the protocol of research.

EQUIPMENT

1. Set for bacteriological research: Tripod - 8 pcs
Tweezers - 8 PC. Bacteriological loop - 8 pcs. Bottle with physical solution - 8 pcs
Spirit lamp - 8 pcs.
2. Microscopes - 8 pcs
Immersion oil 1. A set of paints according to Gram - 8 pcs.
2. cups of environment Endo with growth of intestinal sticks - 8 pcs
3. test tubes of environment Ressel - 8 pcs
4. test tubes with BCH - 8 PC.
5. cups of petri with indicator stripes on indole - 8 PC
6. cups of petri with indicator stripes on hydrogen sulfide - 8 PC
7. Tank drugs

PROTOCOL RESEARCH

No P/P	researched material	results research	Graphic image

METHODOLOGICAL RECOMMENDATIONS

AT connections With difficulty differentiation pathogens intestinal diseases, defiant similar clinical manifestations, necessary conducting integrated microbiological research, including simultaneous Search in researched material pathogens escherichiosis, shigellosis, salmonellosis and cholera.

1. The test material (stools of the patient) is inoculated on the surface of one of the differential diagnostic media for the isolation of the causative agent of intestinal diseases (Wednesday Endo) and 1 %alkaline agar for allocation pathogen cholera.

Sowing held stroke on surfaces dense nutritional environments With goal mechanical separation microbes and receiving isolated colonies.

Cups with 1% alkaline agar are incubated at 37 degrees. 10-12 hours, medium cups Endo - 18-24 hours.

2. After incubation in a thermostat, inoculations on plates with Endo media and 1% alkaline agar are viewed in passing and refractive light. Without any signs of microbial growth on alkaline agar, a negative answer is given for finding pathogen cholera in researched material.

On the Endo medium, after 18-24 hours of growth in a thermostat, the presence of colonies of raspberry- red (fermenting lactose incoming in composition environment) and colorless (not fermenting lactose).

3. Colorless ("suspicious") colonies are sown on Ressel's medium. Medium composition Ressel: MPA, 1 % lactose, 0.1 % glucose and indicator Andrede.

Sowing is done as follows: the removed colony carefully, without touching the edges test tubes are introduced into the condensation liquid, then the entire beveled the surface of the medium and make an injection into the depth of the column. A test tube with seeding on Ressel's medium put in thermostat (37°C) for a day (18-24 h.).

Simultaneously for study proteolytic activity culture lactose-negative colonies sown in test tube with BCH With indicator papers, impregnated acetate lead and oxalic acid for definitions education hydrogen sulfide and indole. test tube put in thermostat (37°C, 18-24 h.)

Escherichiosis (intestinal coliinfection) is an acute intestinal infection caused by various serological groups of enteropathogenic *Escherichia coli* (EPEC), occurring with symptoms of general intoxication and a syndrome of lesions of the gastrointestinal intestinal tract.

Etiology escherichiosis.

pathogens — enteropathogenic intestinal sticks —belong to to mind *Escheirichia*, kind *Escherichia*, family enterobacteraceae, present yourself Gram-negative rods that are stable in the environment. Can be stored for months soil, water, bowel movements. Good are growing on conventional nutritional environments. Quickly perish at boiling and impact disinfectants funds. *Escherichia* have complex antigenic structure: somatic O antigen (thermostable), surface (capsular) K antigen and flagellate H-antigen (thermolabile).

Intestinal infection, caused EPKP, meet more often at children early ageClassification escherichiosis:

- Enteropathogenic (salmonella-like).
- Enterotoxic (cholera-like).
- Enteroinvasive (dysentery-like).
- Enterohemorrhagic.

The diagnosis of escherichiosis can only be established by isolating the pathogen. For

bacteriological research take away feces, emetic masses, flushing water stomach, at generalized forms - blood, CSF. Conduct study bowel movements are needed immediately, as soon as the patient seeks help from a doctor, since with flow time probability allocation pathogen quickly decreases. Collection bowel movements held after natural defecation or With help tampons in test tubes with a glycerin mixture in an amount of not more than 1/3 of the volume of the preservative, and emetic masses and washings of the stomach - in glass jars with a capacity of 200-250 ml. In medical the institution must conduct at least three diagnostic studies (the first - at admission sick before destination to him antibiotics, chemotherapy drugs).

In order to isolate EPEC and ETEC, stool samples should be taken from the last servings, at research EICP - samples With impurity mucus.

Selected material in flow first 2 h deliver in laboratory if this is impossible - placed in a refrigerator and sent to the laboratory no later than 12 hours after fence.

At decision question about etiological roles pathogen at occurrence intestinal infections necessary take account of the following criteria:

- isolation of Escherichia certain serovars related to EPEC, EIEC, ETEC, EHEC or EPEC, in monoculture in combination with non-pathogenic Escherichia serovars; if escherichia pathogenic diagnosis maybe to be installed by alone positive bakposeva;
- massive excretion of ETEC (10⁶/g faeces or more) and their significant predominance above representatives another conditionally pathogenic flora.

Serological research methods have a certain diagnostic value, although they are less informative, they are not convincing, since false positives are possible results due to antigenic similarities With others enterobacteria. Are used for retrospective diagnostics, especially in time outbreaks. AT the present time from serological methods research use RGA (diagnostic titer 1:200 - 1:400 for adults, 1:40 - 1:80 for children); reaction immunofluorescence; reaction immune sorption antibodies, labeled enzymes; reaction neutralization; reaction agglutination with autoculture with an increase in antibody titer by 4 or more times in dynamics diseases.

A promising diagnostic method is the polymerase chain reaction (PCR). To prove pathogenicity escherichia, need to make sure, what she It has receptors providing adhesiveness, maybe produce thermolabile and thermostable toxins, contains plasmid DNA, encoding toxin formation (Protasov S.A., 2003).

If non-pathogenic Escherichia are isolated, it is necessary to approach the diagnosis as such at others OKI, caused conditionally pathogenic flora: triple massive height microorganism, absence sowing pathogenic pathogens.

The diagnosis of "escherichiosis", as noted, is incompetent without bacteriological, as well as serological confirmation. Exception is clinical and epidemiological justification diagnosis.

Instrumental methods surveys (sigmoidoscopy, colonoscopy) at escherichiosis uninformative.

When making the final diagnosis, the type of isolated pathogen is indicated, syndrome of lesions of the digestive tract, the severity of the disease. With a protracted the course also marks the nature of the course of the disease. For example: escherichiosis (E. coli O111) in form acute gastroenteritis, middle degree gravity.

Diagnosis bacteriocarrier can to be installed only in those cases, when clinical symptoms diseases missing in the present time and not noted in previous 1-1.5 months Bacteriocarrier, as a rule, short-term (1-2 times pathogen release). In such cases, when making a diagnosis, only the type is indicated pathogen. For example: bacteriocarrier enteropathogenic Escherichia O125.

Etiology. Pathogen (Yersinia enterocolica) - gram-negative bacillus, anaerobe, grows well on ordinary nutrient media at low temperatures. Known 30 serovars. Illness human more often cause 3rd, 5th, 8th and 9th serovars.

Intestinal yersiniosis.

Epidemiology. source infections are human and animals, sick and carriers. Especially often the pathogen is found in mouse-like rodents, large horned livestock, pigs, dogs, cats, in dairy products, ice cream. Infection human going on through mouth at use infected food, water or contact way.

Disease meets during Total of the year.

Pathogenesis. Pathogen breeds in thin intestines, due to what develops enterocolitis or

gastroenterocolitis. AT heavy cases in areas terminal department thin guts arises ulcerative process With involving mesenteric lymphatic nodes. At penetration pathogen in blood bacteremia is noted and generalization process with development inflammation in organs.

Clinic. The incubation period is 2-3 days. Clinical symptoms in patients practically does not differ from that in pseudotuberculosis. However, it is necessary to have mind that with intestinal yersiniosis, the disease often begins with intestinal disorders (copious watery stools mixed with blood), and damage to internal organs occurs How would secondarily on height of clinical manifestations and more often in heavy cases.

In the diagnosis of intestinal yersiniosis , the leading role is played by bacteriological and serological research methods. Yersinia enterocolica can be isolated from feces, blood, urine, pus, mucus from the pharynx, lymph node. From the methods of serological diagnostics use reaction agglutination and reaction indirect hemagglutination. Diagnostic titer 1:100 and above. More authentic increase in the titer of specific antibodies in dynamics diseases.

Prevention of intestinal yersiniosis is carried out in the same way as for other intestinal diseases. infections. Specific prevention not developed.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #4

THEME: BACTERIA - PATHOGENS INTESTINAL INFECTIONS
(pathogens abdominal typhoid, salmonellosis).

Learning goal: to train students in the methods of microbiological diagnostics and specific prevention intestinal diseases.

PLAN:

5. Taxonomy and main biological properties of pathogens abdominal typhoid, salmonellosis.
6. Epidemiology, pathogenesis, immunity called diseases.
7. Principles microbiological diagnostics abdominal typhoid, salmonellosis.
8. Preparations for etiotropic therapy and specific prevention of typhoid fever, salmonellosis.

INDEPENDENT JOB

1. Accounting for the results on the differential diagnostic environment
Endo, bismuth sulfite agar (demonstration).
2. Accounting for results on the Ressel medium and MPB.
3. Accounting for results reactions
Vidal.

EQUIPMENT

1. Set for bacteriological research Tripod - 8 pcs
Tweezers - 8 PC. Bacteriological
loop - 8 pcs. Bottle With physical

solution - 8pcs

Spirit lamp -8 PC.

2. Microscopes - 8 pcs.

Immersion butter

3. Paint set on Gramu – 8 pcs.

4. test tubes co environment Ressel - 8pcs (accounting result lessons #1)

5. Tubes with BCH - 8 PC. (accounting for the result lessons #1)

6. Tank drugs

7. tables

METHODOLOGICAL RECOMMENDATIONS Typhoid fever - acute cyclic ongoing intestinal anthroponotic infection caused by *Salmonella typhi* bacteria (*Salmonella enterica* serotype typhi), with alimentary transmission (fecal-oral), characterized by fever, symptoms of general intoxication with the development of typhoid status, roseolous rashes on skin, hepato- and splenomegaly and specific

defeat lymphatic systems lower department thin intestines.

Pathogen — *Salmonella Typhi* from families Enterobacteriaceae kind *Salmonellamobile* gram negative wand With rounded ends, Good paintable everyone aniline dyes. Works out endotoxin, pathogenic only for person. Not forms disputes.

The typhoid bacteria is pretty resistant in the environment: in fresh water reservoirs, they persist for up to a month, on vegetables and fruits - up to 10 days, and in dairy products may multiply and accumulate.

Under impact 3 % solution chloramine, five % solution carbolic acid, sublimates (1:1000), 96 % ethyl alcohol they perish through some minutes.

Salmonella abdominal typhus have complex antigenic structure. Various serovars contain characteristic kit antigenic factors which add up from combinations O- and H-antigens.

laboratory diagnostics before Total is in bacteriological examination of blood, feces, urine, bile. The blood culture method can be used from the first days of illness and until the end of the febrile period, preferably before treatment. For of this, 5-10 ml of blood from the cubital vein at the bedside of the patient is inoculated into 20% bile broth or Wednesday Rapoport, meat-peptone bouillon With 1 % glucose, or even in sterile distilled water. Volume environments — 50-100 ml. Ratio material and environments should be 1:10. Feces, urine, duodenal contents are examined from the 2nd week from the beginning diseases, sowing on environments Ploskireva, Levin, Muller and others Preliminary result these research receive through 2 day, final — through four days. For identifying abdominal typhoid sticks in faeces, urine, duodenal content use RIF with labeled sera for O- and Vi-antigens. Preliminary answer maybe to be received in flow 1 h, final — through 5-20 h.

Of the serological methods, RA (Vidal) and RPHA with cysteine are used. reaction Vidal is placed with H- and O-antigens from the 7th-9th day of the disease, repeated on the 3rd-4th week for determining the increase in titer (from 1:200 to 1:400-1:800-1:1600). The last one matters for exceptions positive result reactions, which maybe to be conditioned prior immunization against typhoid fever. The answer can be received through 18-20 hours. When setting up RPHA, the results are recorded after incubation of the plates at 37 ° C for 1.5-2 hours and again after 24 hours at room temperature. Positive counts reaction in titre 1:40 and higher. **salmonellosis** — sharp intestinal infections animals and human, called salmonella. Acute infectious zooanthroponotic disease, called salmonella and characterized, in general, by the development of intoxication and damage to the gastrointestinal intestinal tract.

salmonellosis at human consider How certain disease (nosological form), distinguishing it from typhoid fever and paratyphoid fever. Main source infections - food products, less often - a sick animal, in some cases a source infections maybe to be human (sick or bacteriocarrier). Infection going on through contaminated food, usually of animal origin (meat and meat products, milk, eggs, especially duck and goose), at forced, wrong slaughter animals, violation rules storage and cooking products (contact finished and raw products, inadequate thermal treatment products before

consumption, etc.). Salmonellosis develops when organism fall accumulated in products live salmonella.

On the territory of the Russian Federation, the following serovars of the Salmonella species are most common: enterica subspecies enterica: Salmonella Enteritidis, Salmonella Typhimurium , Salmonella infantis.

Clinical manifestations salmonellosis varied — from asymptomatic carriage of the infectious agent to severe septic forms. Incubation period fluctuates from 2-6 hours before 2-3 days.

There are several clinical forms of salmonellosis:

1. Gastrointestinal the form
2. typhoid the form
3. Septic the form

AT 15-17 % cases salmonellosis in period convalescence observed short-term bacteriocarrier. Possible "transient" carriage (single selection salmonella without clinical manifestations) and chronic bacteriocarrier.

Diagnostics salmonellosis carried out complex With taking into account epidemiological data, symptoms and results laboratory research, aimed at isolation and typing of the pathogen. The main type of typing salmonella is reaction agglutination. For her holding before recent time used hyperimmune sera, but now they have been replaced by monoclonal antibodies to salmonella.

Prevention.

Veterinary and sanitary supervision of slaughter and processing of carcasses; implementation of sanitary rules cooking, storage and implementation food products; examination incoming on work on enterprises public nutrition and trade, children's institutions.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #5

THEME: BACTERIA - PATHOGENS INTESTINAL INFECTIONS
(pathogens shigellosis, cholera).

Target lessons:

1. Train students methods microbiological diagnostics and specific prevention intestinal diseases.

PLAN:

5. Taxonomy and main biological properties pathogens shigellosis, cholera.
6. Epidemiology, pathogenesis, immunity called diseases.
7. Principles microbiological diagnostics shigellosis, cholera.
8. Preparations for etiotropic therapy and specific prevention shigellosis, cholera.

INDEPENDENT JOB

3. Accounting results express diagnostics cholera (demonstration).
4. Accounting for the results of sowing on a differential diagnostic medium Ploskireva (macro- and microscopic research).

EQUIPMENT

1. Set for bacteriological research Tripod - 8 pcs
Tweezers - 8 PC.
Bacteriological loop - 8 pcs. Bottle
With physical solution - 8 pcs
Spirit lamp - 8 PC.
2. Microscopes - 8 pcs.
Immersion butter
3. Paint set on Gramu – 8 pcs.
4. cups co environment Ploskireva With the growth of the colonies 8 pcs
5. Demonstration: triad Field-Yermolyeva - four PC.
6. Demonstration: micropreparations cholera vibrio, shigella
7. Tank drugs
8. tables

METHODOLOGICAL RECOMMENDATIONS

shigellosis — national team group infectious diseases, called bacteria kind shigella (*Shigella*).

Dysentery - shigellosis, occurring with symptoms of intoxication and predominant defeat distal department thick intestines.

Etiology.

pathogens — gram negative motionless (generic sign) bacteria kind *Shigella* family Enterobacteriaceae.

Epidemiology.

The source of infection is sick persons and bacteria carriers. Shigellosis is registered within Total of the year with lifting incidence in warm season.

Mechanisms transmission — fecal-oral and contact household, through water, food products. certain role in dissemination infections play insects- carriers: flies, cockroaches.

infectious dose is 200-300 alive cells, what usually enough for development diseases.

Incubation period lasts 1-7 days.

The pathogenesis of shigellosis. The entrance gate of infection is the intestine, where reproduction shigella. Invasion shigella going on predominantly in enterocytes distal department thick intestines, what leads to destruction enterocytes, development local inflammatory changes in form edema, hyperemia, erosion, superficial ulceration. Endotoxins shigella, hitting in blood, cause general intoxication, up to before development endotoxin shock violation all species exchange substances - protein, fatty, water-salt, with development exicosis various degree.

Treatment

Etiotropic (effect on the pathogen) treatment is carried out with drugs: drugs

nitrofurane series (furazolidone, furadonin),

quinolones (chlorquinaldone),

fluoroquinolones (ciprofloxacin).

Pathogenetic treatment consists of detoxification therapy with isotonic saline solutions (solution Ringer), enterosorbents (enterosorb, Activated coal, Polyphepan, Smecta), as well as vitamin therapy. Carry out the correction of dysbacteriosis. *Laboratory diagnostics*

shigellosis.

1. General blood analysis. Leukocytosis, neutrophilic shift to the left, increased ESR; degree changes usually corresponds gravity states.
2. Bacteriological method. Material for research serve excreta sick and vomit. They use differential diagnostic media (Ploskireva, Endo or Levin).
3. Serological method. Explore paired serum in RPGA With erythrocyte diagnosticum for detection antibodies and growth them titer.

Minimum conditionally diagnostic titer antibodies to diagnosticum shigella Flexner for children before 3rd years old think reaction in breeding 1:100 for the rest diagnosticums 1:200 or 4th multiple titer increase antibodies in disease dynamics.

4. An immunofluorescent method is also used to detect antigen in faeces, urine, blood; reaction growth titra phage (RNF), reaction neutralization antibodies (RNA), enzyme immunoassay method (IFA) and immunoradiometric analysis (IRA).

5. Coprocytological study carry out With first days illness. At microscopic research take into account elevated number leukocytes, erythrocytes, cells intestinal epithelium, Availability starch, fat and products his splitting, cysts protozoa, eggs worms.

Cholera (IC. cholera (Greek cholera, from cholē bile + rheō to flow, expire)) - acute intestinal anthroponotic infection, called bacteria kind Vibrio cholerae. Characterized fecal-oral mechanism infection, defeat thin intestines, watery diarrhea vomiting, fastest loss organism liquids and electrolytes with the development of varying degrees of dehydration up to hypovolemic shock and of death.

Etiology. Known more 150 serogroups Vibrio cholerae; them share on agglutinating typical cholera serum O1 (V. cholerae O1) and on not agglutinating typical cholera serum O1 (V. cholerae non O1) .

"Classic" cholera called cholera vibrio serogroups O1 (Vibrio cholerae O1). Distinguish two biovar (biotype) this serogroups: classical (Vibrio cholerae biovar cholerae) and El Tor (Vibrio cholerae biovar eltor).

By morphological, cultural and serological characteristics they similar: short curved mobile sticks, having flagellum, gram negative aerobes, Good stained aniline dyes, dispute and capsules not form, grow on alkaline media (pH 7.6-9.2) at a temperature of 10-40 °C. Vibrio cholerae El-Thor in difference from classical able hemolyze erythrocytes sheep (not always).

Every from these biotypes on O antigen (somatic) subdivided on serotypes. Serotype Inaba (Inaba) contains fraction C, serotype Ogawa (Ogawa) - faction B and serotype Gikoshima (more correctly Gikoshima) (Hikojima) - fractions A, B and C. H-antigen cholera vibrios (flagellate) — general for all serotypes. cholera vibrios form cholera toxin — proteinaceous enterotoxin.

Vibrio cholerae non-O1 causes cholera-like diarrhea of varying severity, which same maybe end lethal outcome

As example can lead big epidemic caused Vibrio cholerae serogroup O139 Bengal. She started in October 1992 in the Madras Port of South India and, rapidly spreading along the coast of Bengal, reached Bangladesh in December 1992, where only for the first 3 months 1993 caused more how 100000 cases diseases.

Laboratory diagnostics. Purpose of diagnosis: indication of Vibrio cholerae in stool and / or vomit, water, determination of agglutinins and vibriocidal antibodies in paired sera blood sick

Methodology diagnostics. Sowing bacteriological material (excreta, emetic mass, water) on thiosulfate-citrate-bile-salt-sucrose agar (eng. TCBS), as well as on 1% alkaline peptone water; subsequent transfer to the second peptone water and sowing on cups co alkaline agar.

Selection clean culture, identification.

Study biochemical properties of the isolated culture — ability decompose those or other carbohydrates, t. n. "row sugars" — sucrose, arabinose, mannitol.

Reaction agglutination co specific sera

Prevention. Warning drift infections from endemic foci

Compliance sanitary and hygienic measures: disinfection water, the washing up hands, thermal food processing, decontamination of common areas, etc. Early detection, isolation and treatment sick and vibriocarriers

Specific prevention cholera vaccine and cholero-gen toxoid. cholera the vaccine has short (3-6 months) validity.

AT the present time there are the following oral anti-cholera vaccines:

Vaccine WC/rBS — includes from killed whole cells v. Cholerae O1 With purified recombinant B-subunit of cholera toxoid (WC / rBS) - provides 85-90- percent protection in all age groups for six

months after taking two doses With weekly break.

Modified vaccine WC/rBS — not contains recombinant B-subunits. Necessary take two doses this vaccines With weekly break. Vaccine licensed only in Vietnam.

Vaccine CVD 103-HgR — includes from weakened alive oral genetically modified strains of *V. Cholerae* O1 (CVD 103-HgR). Single dose of vaccine provides protection against *V. Cholerae* at a high level (95%). Three months after taking a vaccine protection from *v. Cholerae* El Tor was at the level 65 %.

TIMELINE

1. Definition original level knowledge	----- thirty min.
2. Independent Job	----- thirty min.
3. Examination protocols	----- 10 min.
four. Cleaning working places	----- 10 min.
five. Control final level of knowledge and task on house	----- 10 minutes
	.

PRACTICAL OCCUPATION No. 6

TOPIC: PATHOGENIC ANAEROBES. LABORATORY METHODS DIAGNOSIS, SPECIFIC PREVENTION And THERAPIES ANAEROBIC DISEASES.

TRAINING GOAL:

1. Explore modern methods microbiological diagnostics diseases, called anaerobes.
- 2.To study preparations for specific prophylaxis and therapy of anaerobicdiseases.

PLAN LESSONS

1. Modern ideas about the etiology of anaerobic infection. Clostridial and non-clostridial anaerobic infection.
2. Morphological, cultural and biochemical properties pathogens anaerobic infections: Clostridium (gas gangrene, tetanus, botulism), peptostreptococci, bacteroids, fusobacteria, anaerobic vibrios, campylobacter andspirillum
3. Pathogenetic aspects of anaerobic infection: primary exogenous and secondary, endogenous. Mechanisms occurrence. Opportunistic anaerobic and mixed infections.
4. Methods microbiological diagnostics anaerobic infections.
5. Principles specific prevention anaerobic infections. Preparations foractive and passive immunization.
6. Principles of specific therapy of anaerobic infection. Etiotropic and pathogenetic therapy: antibacterial, hyperbaric oxygenation and etc.

INDEPENDENT JOB

1. Microscopic method for the diagnosis of gas gangrene: the study of a smear-imprint from

- purulent wounds, coloring on Gram.
2. Bacteriological method diagnostics anaerobic infections:
3. th stage - studying on five% bloody agar isolated colonies bacteroids and peptostreptococcus, allocated from purulent exudate.
4. Further- receiving clean culture anaerobic bacteria in semi-liquid environment AS. Demonstration of selective anaerobe culture media: Kitta-Tarozzi, "high" column sugar agar.
5. th stage - identification of a pure culture of anaerobic bacteria by biochemical properties With using test systems AP1-Ap (principle "variegated row").
6. Determination of sensitivity of anaerobic bacteria to antibiotics (micromethod). Demonstration of results sowing net culture in microcassette with antibiotics.
7. Description drugs for specific prevention clostridial anaerobic infection: gas gangrene tetraanotoxin, pentaanotoxin (+ tetanus toxoid), tetanus toxoid component drugs ADS and vaccines DPT, TABte.
8. Description drugs for specific therapy clostridial anaerobic infections: polyvalent antiganangrenous serum, antitoxic tetanus toxoid serum, antitoxic monoclonal and polyvalent anti-botulinum serums.
9. Decor protocol research.

EQUIPMENT

1. Set for bacteriological research Tripod - 8 pcs
Tweezers - 8 PC. Bacteriological
loop - 8 pcs. Bottle With physical
solution - 8 pcs
Spirit lamp - 8 PC.
2. Microscopes - 8 pcs. Immersion butter
- 3 Paint set on Gramu – 8 pcs.
- 4 cups with KA With growth colonies - 8 pcs
- 5 Demo: Kitta-Tarozzi and sugar column- four PC.
- 6 Demonstration: micropreparations pathogenic anaerobes
- 7 Tank drugs
- 8 tables

METHODOLOGICAL RECOMMENDATIONS

4. Microscopic method diagnostics gas gangrene. AT smear- imprint from purulent wounds (coloring on Gram) are found rod-shaped cells purple colors.

5. Bacteriological method diagnostics anaerobic infections.

1-th stage. First day. On 5% blood agar in a Petri dish (after cultivation in anaerostat: 80% N₂ , _ 10% H₂ _ 10% CO₂) determined some species isolated colonies, including those with various types of hemolysis (α , β) and pigment (for example, black pigment bacteroids groups melaninogenicus). **Second day.** AT test tube With clean culture of peptostreptococci in a semi-liquid AS medium, small granules of white colors at the bottom parts of the test tube with the medium. When monitoring the purity of the isolated culture (coloring gentian violet) chains are defined from elongated cocci of blue color.

2-th stage. AT test system API-An for identification pure cultures on biochemical properties determined fermentation glucose (change coloring indicator in yellow) in the absence of other manifestations of glycolytic, as well as proteolytic activity (negative samples on indole and hydrogen sulfide).

3-th stage. At definition sensitivity anaerobic bacteria to antibiotics in microcassette (after cultivation in anaerostC) are celebrated positive and negative options results.

4- stage . At studying ampoules With drugs for specific prevention and therapy anaerobic infections, in protocol are celebrated goals (prevention, treatment), nature of immunization (active or passive, antitoxic or antibacterial), testimony to application and peculiarities use everyone drug.

PROTOCOL RESEARCH

№№ P/P	researched material	results research	Graphic image
1.	Smear-imprint from a festering wound. Coloring on Gram.		

Informational material

tetanus severe wound infection.

Morphology Gram-positive rods with rounded ends. located alone or chain. controversy situated terminal.

Cultural properties obligate anaerobe. On MPA and gelatin in strictly anaerobic conditions pathogen growing slowly and forms thin transparent colonies. When sowing column in semi-liquid agar through 24-48 hours forms colonies in form "lentils" R –forms or "fluff" S forms.

Pathogenicity factors are exotoxins tetanospasmin and tetanolysin.

Antigenic structure – Oh and H antigens.

Immunity. There is no natural immunity in humans to tetanus. Diagnostics: bacterioscopic, bacteriological and biological.

Treatment sent on neutralization tetanus toxin toxoid. Apply tetanus toxoid horse serum in dose 50-100 thousand ME.

Prevention- surgical treatment wounds. creations artificial active immunity in planned okay vaccination DPT, ADSm. Primary vaccination carry out children in 3- monthly age.

Clostridia botulism

Botulism is an acute food poisoning that occurs predominantly defeat central and vegetative systems.

Morphology - rods with rounded ends, movable, peritrichous. controversy situated subterminally.

Cultural properties - strict anaerobes. Grow well on Kitta-Tarozzi media, bouillon from meat fish. calls turbidity environments and gas formation.

Everybody types clostridia botulism form hydrogen sulfide.

The antigenic structure has group-specific (H) flagella and type-specific somatic (O) antigens.

Pathogenicity factors - botulinum toxin is a protein that exhibits neurotoxic action. Botulinum toxin is the strongest poison known to man.

Immunity. Natural immunity human absent.

Treatment. For treatment on Bezredko sick i/v introduce one international medical dose (on 10000 IU serum types AND and E and 5000 IU type C).

Prevention. For emergency prevention used polyvalent (types A, B, E) equine serum.

Clostridia gas gangrene.

Anaerobic wound infection (gas gangrene, anaerobic myositis) - severe wound infection human infection and animals, caused by bacilli kind Clostridium perfringens.

Morphology. Vegetative cells - large, gram-positive, motionless.

Classic forms submitted under direct corner ends. AT body form capsules, they are the most

expressed in virulent strains. Resistant to phagocytosis.

cultural properties. On dense media, *C. Perfiinqens* of type A forms S and R -round colonies. S - dome-shaped colonies, with smooth even edges. R - colonies wrong forms edges; in depth of agar resemble lumps cotton wool.

Growth on liquid and semi-liquid nutrient media, especially those containing glucose, very rapidly with the formation H₂ and CO₂ and usually ends in 8-12 hours. Turbidity of the medium and active gas formation can be observed after 4-8 hours cultivation.

Biochemical activity- splits With education acids and gas glucose, sucrose, maltose lactose mannose starch.

Proteolytic activity weak; liquefies gelatin, intensively curdle milk.

Antigenic structure - Everybody serovars form α - toxin (lecithinase). Pathogen forms at least 12 identifying toxins and enzymes that play a role in pathogenesis gas gangrene.

Clostridium perfiinqens is widely distributed in the environment; it is isolated from water, soil, sewage. Spores can persist in the environment for a long time. environment, capable vegetate in soil. controversy distinguishes high sustainability to chemical and physical influences.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #7

TOPIC: **DIAGNOSTICS OF ZONONOSE** **BACTERIAL** **INFECTIONS**
(tularemia brucellosis)

Educational goal:

1. Train students methods microbiological diagnostics and specific prevention brucellosis, tularemia.

PLAN:

6. Taxonomy and main biological properties of pathogens brucellosis, tularemia.
7. Epidemiology, pathogenesis, immunity called diseases.
8. Principles microbiological diagnostics brucellosis, tularemia
9. Preparations for etiotropic therapy and specific prevention brucellosis.tularemia
10. Sacha module.

INDEPENDENT JOB

3. Setting and taking into account the results of the Hedderson reaction and Wright at brucellosis.
4. staging and accounting results reactions agglutination at tularemia

EQUIPMENT

1. Set for bacteriological research Tripod - 8 pcs
Tweezers - 8 PC. Bacteriological
loop - 8 pcs. Bottle With physical
solution - 8 pcs
Spirit lamp - 8 PC.
2. Microscopes - 8 pcs.
Immersion butter
3. Paint set on Gramu - 8 pcs
4. Finished smears with brucella for coloring on Gramu
5. Demonstration: micropreparations Brucella and pathogen tularemia
6. staging reactions Wright :
7. Test tubes 6 pcs-8 sets
8. Researched serum - 8 PC.
9. brucellosis diagnosticum - 8 PC.
10. pipettes 1.0 - 8 pcs
11. Reaction Hedderson on glass (demonstration)
12. Tank drugs
13. tables

GUIDELINES SEROLOGICAL DIAGNOSIS brucellosis

Serum of patients with brucellosis accumulates agglutinating (initially Ig M, then Ig G), incomplete blockers (Ig A , IgG) and opsonic (IgD) antibodies. For their detection for diagnostic purposes using the reaction of Wright and Hedelson. Reaction agglutination- one from major diagnostic methods at brucellosis.

1. staging reactions Wright held With goal definitions content in blood serum sick specific antibodies. Components reactions:

a) researched serum in breeding 1:25;

b) antigen — suspension killed brucella (diagnosticum Wright).

SCHEME REACTIONS WRIGHT.

No. of tubes	1	2	3	four	five	6	7
Components							
5. Physiological solution	0.5	0.5	0.5	0.5	0.5	0.5	0.5
6. Serumsick (1:25)	0.5	0.5	0.5	0.5	0.5	-	0.5
7. Dilutions serum	1:50	1:100	1: 200	1:400	1:800	-	-
8. Diagnosticum Wright	0.5	0.5	0.5	0.5	0.5	-	-

Accounting results held through 18-20 hours, so on lesson offered demonstration reactions Wright. students carry out results accounting and make a conclusion.

2. staging reactions Hedderson.

Reaction put at mass survey on brucellosis With using glass plates. Components reactions:

- a) undiluted serum blood sick;
- b) antigen - suspension killed and painted crystal violet brucella.

SCHEME REACTIONS HEDDELSON

number of squares	1	2	3	four	Control	
Components					serum	antigen
1. Physiological solution	-	-			0.03	0.03
2. Serumsick	0.08	0.04	0.02	0.01	0.02	-
3. Diagnosticum Wright	0.03	0.03	0.03	0.03	-	0.03

students carry out reaction on one's own and does conclusion.

CONCLUSION:

PROTOCOL RESEARCH

№№ P/P	researched material	results Research	Graphic image

Brucellosis (IC. brucellosis) — zoonotic infection, transmitted from sick animals man, characterized multiple defeat bodies and systems organism person.

Pathogen diseases — group microorganisms kind brucella. pathogenic for There are three human beings: the causative agent of brucellosis in small cattle (*Brucella melitensis*), the causative agent of brucellosis in cattle (*Brucella abortus*), the causative agent of brucellosis pigs (*Brucella suis*).

pathogens brucellosis — bacteria kind brucella — Good tolerate low temperatures and freezing, they remain in water for up to 5 months, in soil - 3 months. and more, in cow's milk - up to 45 days, in cheese - up to 60 days, in butter, cream, yogurt and fresh cheeses — in flow Total period them food values; in frozen meat — St.

five month, in saline skins — 2 month, in wool — before 3-4 months At boiling and pasteurized milk brucella die. Disinfectants kill bacteria in for several minutes.

Most often brucellosis get sick homemade animals (goats, sheep, cows, pigs), while in animals abortions and the birth of a dead fetus are observed. *Brucella* excreted into the environment with milk, urine of sick animals and uterine discharge (in time abortion). causative agents of brucellosis same contained in meat sick animals.

AT organism human brucella penetrate through mucous shells digestive and respiratory tract, a same through damaged skin (abrasions, scratches). A person becomes infected with brucellosis by drinking raw milk from sick people. animals and dairy products prepared from it (cheese, butter, cottage cheese, cheese), and also not enough boiled and fried meC. Infection can also occur in production, related With processing skin and wool, a same at care per sick animals and through objects contaminated with their secretions. Milkmaids are most often ill, calves, shepherds, shepherds, vet. workers, zootechnics.

Incubation period (hidden) continues from one weeks before several months, usually 1-3 weeks. brucellosis is characterized by a variety of clinical symptoms; its course can be of varying severity. The disease begins gradually: appear malaise, insomnia, sometimes irritability, head pain, pain in muscles and joints, declining appetite, temperature rises before 37.1-37.3°. More often brucellosis starts acute: temperature rises before 39— 40° appear chills, weakness, plentiful sweating, sharp pain in muscles stiffness and pain in joints. Characteristically defeat circulatory vessels, nervous systems and boneus- tavnogo apparatus, sometimes there may be mental disorders. The illness lasts an average of 3 month, but maybe drag on before 1-2 years old and more. Persistent residual phenomena after transferred brucellosis may lead to disability. At pregnant women women at brucellosis possible spontaneous miscarriage.

Laboratory the confirmation brucellosis significantly limited topics what brucella relate to dangerous pathogens, selection which maybe be carried out only in special laboratories equipped in accordance with the requirements of prevention. In serological and allergological studies, it must be taken into account that in vaccinated vs brucellosis (grafted groups risk, professionally contacting Withanimals) may to be and enough prolonged time positive results How serological reactions, so especially allergic samples

From serological reactions most informative is reaction agglutination (reaction Wright). Agglutination on glass (reaction Huddleson) for diagnostics not is used, it is proposed to identify individuals to be screened for brucellosis, at massive surveys on epidemiological testimony.

Reaction Heddleson often gives false positive results. AT some degree this is related With cross reactions With nearby antigens (yersinia, pathogen tularemia, anti-cholera vaccination and etc.). Should take account of, what Br. melitensis and Br. abortus cross-react with each other but not with Br. canis, so to detect antibodies to this brucella needs a special diagnosticum, which is not yet available. Perhaps, this one from reasons rare occurrence of this varieties brucellosis.

At acute septic form brucellosis antibodies start come to light on 2nd week diseases and in the future their titer increases. Allergy test becomes positive in end 1st and on 2nd week. At chronic growth patterns titra antibodies often fails to identify. It should be borne in mind that the setting of an allergic test (Burne test) maybe drive to the appearance of antibodies or to an increase in titer. Other serological reactions (RSK, RPGA, OFR) less informative on compared to the reaction Wright and not have significant values. Negative results samples Burne allow exclude brucellosis (per exception HIV-infected at which disappear Everybody reactions HRT).

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #8

THEME: BACTERIA - PATHOGENS CONTACT INFECTIONS
(pathogens Siberian ulcers and plague).

Educational goal:

1. Train students methods microbiological diagnostics and specific Siberian ulcers and plague.

PLAN:

5. Taxonomy and main biological properties pathogen Siberian ulcers and plague
6. Epidemiology, pathogenesis, immunity called diseases.
7. Principles microbiological diagnostics Siberian ulcers and plague.
8. Preparations for etiotropic therapy and specific prevention Siberian ulcers and plague.

INDEPENDENT JOB

1. staging reactions thermoring precipitation on Askoli.
2. Accounting reactions RP on Ascoli and do conclusion.
3. Demo smear from autoclaved pus carbuncle from the patient
Siberian ulcer. coloring by Gram.

EQUIPMENT

1. Set for bacteriological research Tripod - 8 pcs
- Tweezers - 8 PC. Bacteriological
- loop - 8 pcs. Bottle With physical
- solution - 8 pcs
- Spirit lamp - 8 pcs. 2.

Microscopes - 8 pcs.

Immersion butter

5. Paint set on Gramu - 8pcs

6. test tubes With anthracoid for coloring by Gram– 8pcs

7. Reaction Ascoli:

8. Pasteur pipettes

9. Precipitinogen -four PC.

10. Narrow test tubes With immune serum

11. Narrow test tubes with normal serum - four PC.

12. Demonstration: micropreparations pathogen Siberian ulcers and plague

13. Tank drugs

14. tables

METHODOLOGICAL RECOMMENDATIONS

Anthrax bacilli are very large (6-10 microns) Gram-positive rods. with chopped ends, in a smear from pure culture are located in short chains (streptobacilli). motionless, form located centrally controversy, a same capsules.

Cultural properties: Anthrax bacilli are aerobes. grow well on simple nutrient media, at a temperature of 12-45 C. On liquid media, they give near-bottom growth in form lump cotton wool; on dense media form large, With uneven edges, rough matte colonies under magnifying glass colonies remind mane lion or head jellyfish. On environments containing 0.05- 0.5 U/ml penicillin, through 3-6 h growth anthrax bacilli form spheroplasts, located chain and reminiscent of in smear pearl necklace .

Biochemical properties: Ferments to acid glucose, saccharose, maltose, starch, inulin; have proteolytic and lipolytic activity. Highlights gelatinase, show low hemolytic, lecithinase and phosphatase activity.

Antigens and factors pathogenicity: Contain generic somatic polysaccharide and specific protein capsular antigens. Produce protein exotoxinpossessing antigenic properties and consisting of several components (lethal, protective and defiant edema). pathogenic for human and many animals.

Resistance: The vegetative form is unstable to environmental factors, but disputes extremely resistant and stored in environment decades, withstand boiling and autoclaving. anthrax bacilli sensitive to penicillin and other antibiotics; spores are resistant to antiseptics and disinfectants. Sporicidal effect possess activated solutions chloramine, hot formaldehyde, peroxides hydrogen.

Epidemiology and pathogenesis: A source infections - sick animals. More often cattle: sheep, goats, horses, deer, buffaloes, camels, pigs. Person is biological a dead end. For Siberian ulcers characteristically multiplicity mechanisms, ways and factors of transmission. A person becomes infected mainly by contact, less often alimentary, aerogenic, etc. when caring for sick animals, slaughter, processing animal raw materials, use meat and others livestock products. Susceptibility to the pathogen relatively low.

The entry gates of infection in most cases are damaged skin, much less often the mucous membranes of the respiratory tract and the gastrointestinal tract. AT pathogenesis is based on the action of the exotoxin of the pathogen, some fractions of which cause protein coagulation, tissue edema, lead to the development of infectious-toxic shock.

Clinical picture: There are skin, pulmonary and intestinal forms of Siberian ulcers. At skin form on place implementation pathogen appears characteristic anthrax carbuncle (hemorrhagic-necrotic inflammation of the deep layers of the skin with skin necrosis and the formation of a brown-black crust), this form is accompanied by edema. Pulmonary and intestinal forms relate to generalized forms and expressed hemorrhagic and necrotic defeat relevant organs.

Duration incubation period - from several hours before 8 days in average 2-3 days.

Generalized forms in one hundred% cases end fatally.

Microbiological diagnostics: Material for research serve content carbuncle, sputum, cal, blood, urine. Microbiological diagnostics carried out in compliance with safety regulations, as in especially dangerous infections. For diagnostics apply Everybody five methods microbiological diagnostics. smears stain by gram, and to detect capsules - according to Ramanovsky-Giemsa, dispute - according to Ozheshka. For allocation clean culture researched material sow on meat-peptone agar and meat-peptone broth, and also infect laboratory animals (white mice maritime pigs). Dedicated clean culture identify on generally accepted scheme, taking into account morphology, the nature of growth on MPA and BCH, biochemical and cultural properties. Anthrax antigens define in REEF and reactions thermoprecipitation according to Ascoli also examine animal corpses, skin and products from it, skins, fur, wool and others products from animal raw materials.

Treatment: Apply antibiotics and anthrax immunoglobulin.

Prevention: For specific prevention use live anthrax vaccine STI (sanitary institute). For emergency prevention appoint anthrax immunoglobulin.

Plague (IC. pestis - infection) - an acute natural focal infectious disease group of quarantine infections, proceeding with an exceptionally severe general condition, fever defeat lymph nodes, lungs and others internal bodies, often With development of sepsis. The disease is characterized by high mortality and extremely high infectiousness.

Plague wand (IC. Yersinia pestis) is a bacterium discovered in 1894 at the same time two scientists: French Alexander Yersin and Japanese Kitasato Shibasaburo.

Incubation period lasts from several hours before 3-6 days. Most The most common forms of plague are bubonic and pneumonic. Mortality in bubonic form plague reached 95%, with pulmonary - 98-99%. Currently with proper treatment mortality is 5-10 %.

The well-known plague epidemics, which claimed millions of lives, left a deep mark on stories humanity.

The causative agent of plague is resistant to low temperatures, well preserved in sputum, but at a temperature of 55 ° C dies within 10-15 minutes, and when boiled - almost immediately. Enters the body through the skin (with a flea bite, usually *Xenopsylla cheopis*), mucous shells respiratory ways, digestive tract, conjunctiva.

By main carrier natural foci plague subdivide on ground squirrels, marmots, gerbils, voles and pikas. In addition to wild rodents, in the epizootic the process sometimes includes the so-called synanthropic rodents (in particular, rats and murine), as well as some wild animals (hares, foxes), being the object hunting. From domestic animals plague get sick camels.

AT natural hearth infection usually going on through bite fleas, previously fed on sick rodent. At bite infected plague bacteria fleas at human on place bite maybe arise papule or pustule, filled hemorrhagic content (skin the form). Then process spreads on lymphatic vessels without manifestation of lymphangitis. Reproduction of bacteria in macrophages lymphatic nodes leads to them sharp increase, merger and education conglomerate (bubonic form). Further generalization of an infection that is not strictly mandatory, especially in the context of modern antibiotic therapy, may lead to the development of a septic form, accompanied by a lesion practically all internal organs. However With epidemiological positions the most important role play

"screenings" of infection in the lung tissue with the development of the pulmonary form of the disease. Since development of plague pneumonia, a sick person himself becomes a source of infection, but with In this case, the pulmonary form of the disease is already transmitted from person to person - an extremely dangerous one, with very fast flow.

Establishing an accurate diagnosis must be carried out using bacteriological research. The material for them is a punctate of a festering lymph node, sputum, blood sick, detachable fistula and ulcers.

laboratory diagnostics carried out With help fluorescent specific antisera, which stain smears detachable ulcers, punctate lymphatic nodes, culture, received on bloody agar.

First vaccine vs plague created in early XX century Vladimir Khavkin.

The treatment of plague patients is currently reduced to the use of antibiotics, sulfonamides

and therapeutic anti-plague serum. Prevention of possible foci disease is to carry out special quarantine measures in port cities, deratization all ships, which walk international flights, creation special anti-plague institutions in steppe areas, where found rodents, detection of plague epizootics among rodents and their control. Disease outbreaks so far since meet in some countries Asia, Africa and South America.

TIMELINE

1. Definition original level knowledge	-----	thirty min.
2. Independent Job	-----	thirty min.
3. Examination protocols	-----	10 min.
four. Cleaning working places	-----	10 min.
five. Control final level knowledge and task on house	-----	10 minutes.

PRACTICAL OCCUPATION #9

THEME: Diagnosis of rickettsiosis, chlamydia, mycoplasmosis, ehrlichiosis

Educational goal:

1. Train students methods microbiological diagnostics and specific prevention of typhus, chlamydia, mycoplasmoses, ehrlichiosis

PLAN:

1. Taxonomy and main biological properties of chlamydia, mycoplasmoses, ehrlichiosis causative agents typhoid,
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles of microbiological diagnosis of typhus , chlamydia, mycoplasmoses, ehrlichiosis

Preparations for etiotropic therapy and specific rash prevention typhoid, chlamydia, mycoplasmoses, ehrlichiosis

INDEPENDENT JOB.

1. staging reactions binding complement at loose typhus With two antigens (from Rickettsia Provachek and Muser). Accounting for results RSK (demonstration).
2. Staging reaction agglutination in typhus (Weyl reaction-Felix-k proteus OH19). Accounting results RA (demonstration).
3. staging RNGA at loose typhus (With erythrocyte antigen).
4. staging RPGA With erythrocyte diagnosticum at plague. Accounting results RPGA (demonstration).
5. staging reaction immunofluorescence (demonstration).
6. Microscopy of finished preparations with the causative agent of chlamydia, mycoplasmosis, ehrlichiosis and rickettsia.

EQUIPMENT

1. Accounting RSC results (demonstration).
2. Accounting results RA (demonstration).

3. Accounting for results RPGA (demonstration).

5. staining reaction immunofluorescence (demonstration).

6. Microscopy of finished preparations with the causative agent of chlamydia, mycoplasmosis, ehrlichiosis and rickettsia.

METHODOLOGICAL RECOMMENDATIONS

Typhus is a group of infectious diseases caused by rickettsiae acute infectious disease transmitted from a sick person to a healthy person through lice.

Epidemiology. At present, the high incidence of typhus has persisted only in some developing countries. However, the long-term preservation of rickettsia in previously ill with typhus and the periodic appearance of relapses in the form of a disease Brill-Zinsser not Maybe at deterioration social conditions (increased migration population, pediculosis, worsening nutrition and etc.).

source infections is sick human, beginning With recent 2-3 days incubation period and up to the 7-8th day from the moment of normalization of body temperature. After this, although rickettsia may for a long time persist in body, convalescent already danger for surrounding not is. Synony typhus transmitted through lice, mainly through clothes, less often through head ones. After feeding on the patient's blood louse becomes contagious through 5-6 days and before end life (then eat 30-40 days). Infection human going on through rubbing faeces lice in damage skin (in combinD). known cases infections at transfusion blood, taken at donors in last days of incubation period. Rickettsia circulating in North America (R. canada), transmitted ticks.

Endemic loose typhus

Endemic typhus (rat, flea, or American typhus) caused by rickettsiae *R. mooseri*. Approximately 40 cases are reported in the US each year. diseases. It meets in regions With relatively warm climate in both hemispheres predominantly in summer and in mostly among rural residents; leaks easier, how epidemic typhoid. it illness chief way rats, which transmitted to humans when bitten by rat fleas. Therefore, the fight against rats is extremely important as preventive measure.

Epidemic loose typhus

Epidemic typhus, also known as classical, European or lousy loose typhus, shipboard or prison fever, called rickettsiae *Provahek*.

The causative agent is a Gram-negative small non-motile bacterium *Rickettsia prowazeki*. It does not form spores and capsules, it is morphologically polymorphic: it can look like cocci, rods; all forms remain pathogenic. Usually they are stained according to the Romanovsky-Giemsa method. or silver plating according to Morozov. Cultivated on complex nutrient media, in chicken embryos, in the lungs of white mice. Reproduce only in the cytoplasm and never in the nucleus infected cells. Possess somatic thermostable and type-specific thermolabile antigen, contain hemolysins and endotoxins. in lice excrement, getting on clothes saves viability and pathogenicity in for 3 months and more. At temperature 56 °C dies per 10 min, at one hundred °C - per thirty With. Quickly inactivated by the action of chloramine, formalin, lysol, acids, alkalis in ordinary concentrations. Assigned to second group pathogenicity.

Pathogenesis.

The gates of infection are minor skin lesions (usually scratchinD), after 5-15 min rickettsia penetrate into the blood. Reproduction of rickettsia occurs intracellularly in endothelium vessels. it leads to swelling and desquamation endothelial cells. Caught in current blood cells are being destroyed released at this rickettsia infect new endothelial cells. The most rapidly breeding process of rickettsia happening in recent days incubation period and in first days fever.

Diagnosis sporadic cases in elementary period disease (before appearance typical exanthema) very difficult. Serological reactions become positive same only from 4-7 days from the onset of the disease. Easier diagnosis during outbreaks epidemiological data (intelligence about incidence, availability lice, contact with patients with typhus, etc.). When an exanthema appears (that is, from the 4th-6th day disease), a clinical diagnosis is already possible. The timing of the appearance and nature of the rash, hyperemia face, enanthema Rosenberg, spots Chiari-Avtsyna, changes co sides nervous systems

- all this allows you to differentiate primarily from typhoid fever (gradual Start, lethargy sick, changes co sides bodies digestion, more late appearance of exanthema in the form of a roseolo-

papular monomorphic rash, the absence petechiae and etc.).

It is necessary to differentiate from other infectious diseases that occur with exanthema, in particular, with other rickettsiosis (endemic typhus, tick-borne rickettsiosis of North Asia, etc.). Some differential diagnostic value has a blood picture. Typhoid fever is characterized by moderate neutrophilic leukocytosis with stab shift, eosinopenia and lymphopenia, moderate increase ESR.

For confirmation diagnosis use various serological reactions. Retained some value Weyl-Felix reaction - agglutination reaction with *Proteus* OXig, especially with an increase in antibody titer during the course of the disease. The most commonly used RSC rickettsial antigen (cooked from rickettsia Provachek), diagnostic titer is considered to be 1:160 and above, as well as an increase in antibody titer. Others also use serological reactions (reaction microagglutination, hemagglutination and etc.).

In the memorandum of the WHO meeting on rickettsiosis (1993), as a recommended diagnostic procedures recommended indirect reaction immunofluorescence. AT sharp phase disease (and period convalescence) antibodies connected With IgM, what used for differences from antibodies in result previously transferred illness. Antibodies begin to be detected in the blood serum from the 4-7th day from the onset of the disease, the maximum the titer is reached after 4-6 weeks from the onset of the disease, then the titers slowly decrease. After transferred loose typhus rickettsia Provacheka in flow many years old persist in the body of a convalescent, this leads to a long-term preservation of antibodies (associated with IgG same in flow many years old, although and in low credits). AT last thing time With diagnostic purposes use trial therapy with tetracycline antibiotics groups. If, when prescribing tetracycline (at usual therapeutic doses), after 24-48h does not occur normalization of body temperature, it allows to exclude typhus (if fever not related With any complication).

Main *etiotropic drug* in the present time are antibiotics tetracycline group, with intolerance to them, levomycetin also turns out to be effective (chloramphenicol).

For *prevention* loose typhus big meaning It has struggle co lice, early diagnostics, insulation and hospitalization sick loose typhus, needed careful sanitization of patients receptionist peace of the hospital and pest control clothes sick. For *specific prevention* used inactivated formalin vaccine containing killed Rickettsia Provachek. Vaccines have been used in time of increased incidence and were effective. Currently available active insecticides, effective methods etiotropic therapy and low incidence meaning anti-typhoid vaccination decreased significantly.

Cause **urogenital chlamydia** are chlamydia - gram negative bacteria, which lost some important mechanisms workings metabolic energy. This defect conditions their intracellular height, thanks to whom they have access to energy-rich metabolic intermediates. They are divided into two species - Chlamydia trachomatis, which combines human pathogens, and Chlamydia psitaci, which includes related microorganisms that primarily affect mammals and birds. Together they form genus Chlamydia, representatives whom possess bacteria-like morphological characteristics and unique cycle development.

Chlamydia in process reproductions undergo row successive changes. infectious particle is yourself small cage (elementary corpuscle) diameter about 0.3 micron With electron dense nucleoid. This particle penetrates in host cell during phagocytosis. From the surface membranes of the cell host around this small particles formed vacuole. Petite particle turns in large (reticular corpuscle), diameter 0.5-1.0 micron, which deprived electron dense nucleoid. Inside educated membrane vacuoles large particle increases and repeatedly divided by formation of a transverse septum. In the end account all vacuole is filled small particles, formed from major calf at them transverse division, and turns in "inclusion" in cytoplasm cells owner. newly formed small particles may go out from cells host and infect new cells. Cycle breeding chlamydia implemented at them interaction With sensitive cell and takes 24-48 h.

Chlamydial infection at men and women most often It has incubation period from 5-7 before thirty days. She maybe call various pathologies.

In men, the urethra is primarily affected, and then other organs. (prostate gland, seminal vesicles, appendages). Canal is more commonly affected in women cervix, after which an ascending infection may occur that captures the uterus, the fallopian tubes, ovaries, a same peritoneum.

Chlamydia not are representatives normal microflora person. Them detection indicates an infectious process, and the absence of clinical symptoms diseases defines only temporary equilibrium between parasite and host in conditions that limit the reproduction of a pathogenic intracellular microorganism, butnot obstructing to him.

Clinically asymptomatic chlamydial infection not less dangerous how her manifest forms, and requires medical and preventive events.

Various methods are used **to detect chlamydial infection , such as direct** definitions pathogen, So and indirect serological examinations.

The material for research in urogenital chlamydia are smears, scrapings from the mucous membrane of the urethra, cervical canal, cervix, rectum, conjunctiva, which take away special spoon special tampons brushes or platinum loop. The sampling of material is the most important stage of diagnostics. When testing for chlamydia by culture, patients should not use antibiotics and other drugs active against chlamydia for a month. If are used cytological methods, drugs it is forbidden apply per 2 weeks before research.

cultural method identifying chlamydia - "gold standard" - is most informative (100% sensitivity), but due to the high cost and complexity is widespread. This method is very important if long-term infection.

Cytological method is in microscopic research superficial scrapings epithelial cells, taken from urethra, cervical channel and others mucous shells With goal discover chlamydia. AT cooked smears, which predominantly stain, determine the presence of specific cells in cellular elements chlamydial inclusions. These intracellular inclusions are more often detected with fresh and untreated infection. The method is simple, accessible, but not sensitive enough; allows the diagnosis of chlamydia infection not more how at 15-20% sick.

Immunofluorescent method - staining chlamydial antigens immunofluorescent dyes on basis monoclonal antibodies. His disadvantage is subjectivity estimates results.

The polymerase chain reaction (PCR) method in the diagnosis of chlamydial infection is method definitions specific site DNA With help DNA analyzer . He has very high sensitivity and specificity.

Serological method identifying chlamydia - detection antichlamydial antibodies in blood . In acute infection diagnostic value is the detection of chlamydial immunoglobulin M (IgM) -antibodies or 4-fold increase in immunoglobulin G titers (IgD) in dynamics after 2 weeks. Medium and low titers of antibodies to chlamydia, as a rule, characteristic of chlamydial cells absorbed by *Trichomonas vaginalis* (during treatment destruction of the trichomonas cell occurs and a new one enters the extracellular spaceportions of chlamydia, which, in turn, stimulate the production of antibodies in the body). It is forbidden With confidence declare about infected chlamydia only on basis availability antichlamydial antibodies. Only combination various methods (not less 2 simultaneously and one from them PCR) gives necessary accuracy diagnostics urogenital chlamydia for both primary diagnosis and control cured.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #10

CHANGE MODULE ON TOPIC: INTestinal infections pathogenic anaerobes, zoonoses, rickettsiosis, chlamydiosis, mycoplasmosis, erlichiosis

PRACTICAL OCCUPATION #11

THEME: Laboratory diagnostics diphtheria and whooping cough.

Educational goal:

1. Teach students the methods of microbiological diagnostics and specific prevention diphtheria, whooping cough

PLAN:

1. Taxonomy and main biological properties pathogens diphtheria, whooping cough
2. Epidemiology, pathogenesis, immunity caused diseases.
3. Principles microbiological diagnostics diphtheria, whooping cough
4. Drugs for etiotropic therapy and specific prevention diphtheria, whooping cough

INDEPENDENT JOB

1. Cooking smear and coloring on method Neisser.
2. Cooking smear and coloring on method Gram.
3. Definition of toxigenicity diphtheria cultures on Ouchterlony.
4. Carrying out tests for cystinase and urease of diphtheria and false - diphtheria sticks.

EQUIPMENT

1. Set for bacteriological research Tripod - 8 pcs
Tweezers - 8 PC. Bacteriological
loop - 8 pcs. Bottle With physical
solution - 8 pcs
Spirit lamp - 8 PC.
2. Microscopes - 8 pcs.
Immersion butter
3. Paint set on Gram - 8 pcs
4. Paint set on Neisser - 8 pcs.
5. Tubes with diphtheroid - 8 pcs
6. Demonstration: toxigenicity diphtheria cultures on Ouchterlony.
7. Demonstration: micropreparation pathogen diphtheria.

8. Demonstration: samples pisa, samples Zaks
9. Tank. drugs
10. Tables.

METHODOLOGICAL RECOMMENDATIONS

diphtheria wand (*Corynebacterium diphtheriae*) — gram-positive rod-shaped bacteria kind *Corynebacterium*. First pathogen was discovered on sections of films obtained from the oropharynx of patients in 1883 by Edwin Klebs (German: Edwin Klebs, 1834-1913). A year later, Friedrich Löffler (German: Friedrich August Johannes Löffler, 1852-1915) a pure culture was isolated. Diphtheria toxin was obtained by E. Ru and A. Yersin (1884-1888 gg.). Anatoxin discovered Ramon Gaston in 1923 G. and offered use it for active immunization. *Corynebacterium diphtheriae* - large (1-8 × 0.3-0.8 microns) straight, slightly curved polymorphic rod-shaped bacteria. At the poles cells, metachromatic grains of volutin are localized, giving the cells a characteristic form "maces". grains volutin stained methylene blue on Neisser. On micropreparations are located alone or due to features division cells are located in form of latin letters V or Y. Dispute and capsules do not form.

Epidemiology. The source of infection in diphtheria are people - sick or healthy carriers toxigenic diphtheria microbes. the greatest epidemic danger present sick diphtheria pharynx, nose and larynx, actively highlighting pathogens into the environment with exhaled air. Minor in this relation meaning play sick diphtheria eye, skin, wounds and others localizations capable of spreading the infection by contact (through hands, objects life).

Pathogenesis. Entrance gates of pathogens of diphtheria can be almost everything areas of integument (skin and mucous membranes) of the macroorganism. However, most often they are mucous shell oropharynx, much less often - larynx, nose, conjunctiva genital bodies, wound surface, skin and others Toxigenic corynebacteria fixed on tissue cells, multiply and in the process of life produce exotoxin, having a local and general effect, causing almost all manifestations pathological process. microbial cells outside fabrics, being the gateway infection, usually, does not apply to direct participation in defeat macroorganism not accept.

diphtheria exotoxin includes from several factions, each from which has independent biological action. One from them - hyaluronidase: destroys hyaluronic acid capillary and raises them permeability. it leads to exit per limits vessels liquid parts blood, impregnation affected fabrics plasma containing fibrinogen along with other components. The second is necrotoxin - causes necrosis of the epithelium at the site of the gate infection, accompanied by a release from epithelial cell thrombokinase. The latter promotes the conversion of fibrinogen into fibrin and education on surfaces affected fabrics fibrinous films. Palatal tonsils, unlike other organs, are covered with multi-row epithelium. As a result the fibrin film formed during diphtheria penetrates deep into the epithelial cover and tight soldered to tissues. Third faction diphtheria toxin - true diphtheria toxin (its main component) is able to displace from cellular structures cytochrome B and thus block the processes of cellular respiration and synthesis in them protein molecules. Most sensitive to this change are myocardium, capillaries and nervous cells. AT cardiomyocytes develop phenomena myocardial dystrophy with their subsequent necrosis, myolysis and the development of infectious-toxic myocarditis. Defeat capillaries at diphtheria accompanied infectious-toxic shock. Damage to nerve cells is accompanied by dystrophic changes in the Schwann cells and demyelination nervous fibers. Along With marked general action diphtheria toxin appears phenomena general intoxication.

Basis laboratory diagnostics constitute bacteriological research: selection pathogen from hearth inflammation, definition his type and toxigenicity. Material take away sterile wadded tampons dry or wetted (before sterilization!) five% solution glycerin. At storage and transportation tampons protect from cooling and drying. The material should be sown no later than 2-4 hours after taking. In patients with angina who were in contact with patients with diphtheria, as well as in persons with typical clinical manifestations of diphtheria, the diagnosis is made even with a negative bacteriological research.

Auxiliary meaning It has definition credits antitoxic antibodies in paired sera at staging

RNGA. toxin formation reveal using RNGA With antibody erythrocyte diagnosticum. For identifying diphtheria toxin proposed use PCR.

Main in treatment diphtheria think introduction antitoxic diphtheria serum. She neutralizes toxin, circulating in blood, Consequently, renders largest Effect at early application

Preventive Events. Vaccination remains main way control diphtheria. Immunization schedule children provides immunization DTP vaccine starting from 3 months of life (vaccinated 3 times with an interval of 30-40 days). Revaccination is carried out 9-12 months after the completed vaccination. For revaccination in 6-7, 11-12 and 16-17 years old apply ADS-M. AT individual cases, for example at contraindications to whooping cough component DPT, ADS-M apply and for vaccination.

Whooping cough (whooping-cough - English; Keuchhusten - it; Coqueluche - French) and paraptussis - sharp infectious disease, clinically indistinguishable from pertussis. Characterized sharp respiratory ways and attacks spasmodic cough.

The causative agent of whooping cough (*Bordetella pertussis*) is a short stick with rounded ends (0.2-1.2 μ m), gram negative motionless, Good stained with aniline dyes. Antigenically heterogeneous. Antigen, which causes the formation of agglutinins (agglutinin), consists of several components. They are named factors and are designated figures from 1 before fourteen. Factor 7 is generic, factor 1 contains *C. pertussis*, fourteen - *C. paraptussis*, rest found in different combinations; for the causative agent of whooping cough, these are factors 2, 3, 4, 5, 6, for paraptussis - 8, nine, 10. Reaction agglutination With adsorbed factorial sera allows differentiate kinds *Bordetella* and determine them antigenic options. pathogens whooping cough and paraptussis very unstable in external environment, so seeding is necessary do immediately after taking the material. Bacteria die quickly when dried, ultraviolet irradiation, under the influence of disinfectants. sensitive to erythromycin, chloramphenicol, antibiotics tetracycline groups, streptomycin.

Pathogenesis. Gates infections is mucous shell respiratory tract. whooping cough microbes attached to cells flickering epithelium, where they multiply on surfaces mucous shells, not penetrating in blood flow. On place implementation pathogen develops inflammatory process, oppressed activity ciliary apparatus cells epithelium and increases secretion mucus. AT further there is ulceration of the epithelium of the respiratory tract and focal necrosis. Pathological process most pronounced in bronchi and bronchioles, less pronounced changes develop in trachea, larynx and nasopharynx. Mucopurulent cork up clearance small bronchi, develops focal atelectasis, emphysema. Observed peribronchial infiltration. AT genesis convulsive seizures It has meaning sensitization organism to toxins pertussis sticks. Permanent irritation receptors respiratory ways conditions cough and leads to formation in respiratory center hearth arousal type dominants. Due to this typical seizures spasmodic cough may to be caused and non-specific irritants. From the dominant focus, excitation can radiate to other departments of the nervous system, for example, vasomotor (increased blood pressure, vasospasm). irradiation arousal explained same appearance spasmodic muscle contractions faces and torso, vomiting and others symptoms whooping cough Postponed whooping cough (How and pertussis vaccinations) does not provide lifelong immunity, so possible repeated diseases whooping cough (about five% cases whooping cough account for on adults people).

Reliable diagnosis in catarrhal period maybe to be staged after receiving results bacteriological research. foundation for research in these cases usually serve epidemiological data (contact With sick whooping cough lack of data on vaccinations, etc.). In the period of spasmodic cough, the diagnosis of whooping cough put much easier, So How appear typical attacks. However need to take into account that sometimes coughing fits similar to whooping cough may be due to others reasons (adenoviral infection, viral pneumonia, compression respiratory tract in malignant neoplasms, infectious mononucleosis and etc.), on the other hand, whooping cough can occur atypically without characteristic seizures (in vaccinated children, in adults). The main method of laboratory confirmation of the diagnosis is selection pathogen whooping cough Frequency allocation depends from timing taking material; in the 1st week of the disease, positive results can be obtained in 95% patients, on the 4th - only 50%, and starting from the 5th week, the microbe can no longer be isolated. Material from nasopharynx take dry swab With immediate sowing on cups

With selective nutritional environment. use same method "cough records", at which cup petri With nutritional environment installed front mouth coughing child (at a distance of about 10 cm), held in this position for several seconds, to catch 5-6 cough shocks. Cup With sowing quickly close lid and put into the thermostat. At transportation is protected from cooling (wrapped in paper, cotton wool, a heating pad filled with hot water is placed in the container). However, in terms of frequency allocation pathogens whooping cough method "cough records" much inferior taking material swab. Serological methods can use for retrospective diagnostics, a same at sick With negative results bacteriological research. From old methods can use RSK, RPGA, agglutination reaction. A 4-fold increase in antibody titers is considered diagnostic. more, a same high titers antibodies (1:80 and higher).

Recently, enzyme immunoassay has been successfully used to detect antibodies in serum (immunoglobulins class M) and in nasopharyngeal slime (immunoglobulins class A). These antibodies appear co 2nd-3rd weeks disease and persist in flow 3 months

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level of knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #12

THEME: LABORATORY DIAGNOSTICS OF TUBERCULOSIS And LEPRAS

Educational goal:

1. Train students methods microbiological diagnostics and specific prevention tuberculosis.

PLAN:

1. Taxonomy and main biological properties pathogens tuberculosis.
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles microbiological diagnostics tuberculosis.
4. Preparations for etiologic therapy and specific prevention tuberculosis.

INDEPENDENT JOB

1. microscoping micropreparations: mycobacteria tuberculosis, meningococci.
2. Explore scheme laboratory diagnostics tuberculosis.
3. will study method microculturing for express diagnostics tuberculosis;
4. microscoping and sketch demonstration a drug "microculture myc. Tuberculosis."

EQUIPMENT

1. Demonstration: microcultures according to Price
2. Demonstration: micropreparations mycobacteria tuberculosis
3. Tank. drugs
4. tables

METHODOLOGICAL RECOMMENDATIONS

Mycobacteria belong to the Mycobacteriaceae family, Mycobacterium genus. Distinguish:

- 1) Mycobacteria human type (Mycobacterium tuberculosis), 2) mycobacteria bovine type (Mycobacterium bovis), 3) mycobacteria avian type (Mycobacterium avium), 4) atypical mycobacteria, which are divided into four groups (according to Runyon):

- a) photochromogenic bacteria, which are growing in darkness in flow 21-46 days in form non-pigmented colonies, but after lighting daytime or electric light acquire yellow or orange coloration. pathogenic for people;
- b) scotochromogenic bacteria, which grow slowly (60-100 days), form yellow- Orange pigment in darkness. Some from them pathogenic: cause defeat lymphatic nodes and lungs at children;
- c) non-photochromogenic Mycobacteria do not form pigment either in the light or in the dark. pathogenic for person;
- d) fast-growing mycobacteria, grow during several days in the form of pigmentless colonies. To this group relate How potentially pathogenic mycobacteria, So and saprophytes.

Pathogen tuberculosis: Mycobacterium tuberculosis is yourself thin, slightly curved sticks long 2.5-3.5 micron, different big polymorphism: long, branched and granular forms. Mycobacterium bovis - short, thick sticks, Mycobacterium avium - filiform, branched forms.

For productions microbiological diagnosis use microscopic, bacteriological, biological, serological and allergic research methods. A wide variety of material can be received for research, depending on whether where the pathological process is located: with pulmonary tuberculosis - sputum, with tuberculosis kidneys - urine, at tuberculosis meningitis - spinal liquid

VI. MICROSCOPIC METHOD

researched material: sputum, urine, spinal liquid. For "enrichment" sputum wide are used methods homogenization and flotation. At staining of smears according to Ziehl-Neelsen, tubercle bacilli are stained bright red Colour. The use of fluorescent microscopy increases the number of tubercular sticks.

Microscopic examination is indicative and allows us to judge only about the presence of acid-resistant bacteria in the material without determining their species and type accessories.

VII. BACTERIOLOGICAL STUDY

Peculiarities method

For release from accompanying microflora researched material handle 10% sulfuric acid or 4-6% solution caustic sodium, a then centrifuged. acid neutralized and the material is poured into several test tubes with environment Lowenstein-Jensen or others special Wednesdays.

Crops are incubated at 37 °C 4-6 weeks or more, since tubercle bacillus breeds very slowly, especially in first generations. Colonies have view greyish or light cream wrinkled or crumbly dry raid.

At identification more often Total define ability dedicated culture synthesize nicotine acid - niacin try Konno - With help which it is possible to distinguish M.tuberculosis, which synthesize nicotinic acid well, from rods M.bovis, forming it in minimal quantities. Atypical mycobacteria have high catalase activity. Peroxidase activity at them not is revealed. Determination of the thermal stability of catalase makes it possible to differentiate virulent for human mycobacteria (human and bovine types), in which it is thermolabile, from acid-resistant saprophytes and atypical mycobacteria, which form thermostable catalase.

For accelerated diagnosis of tuberculosis, the Price microculture method is used. For To do this, thick smears of the material under study are made on several glass slides. smears handle 2-6 % sulfuric acid and neutralize alkali. After this them placed in vials with hemolyzed citrated blood. After 7-14 days material stain on Ziel-Nielsen and microscopic - virulent strains form microcultures, cord-like or braid (Availability cord factor).

VIII. BIOLOGICAL METHOD

Applies With goal allocation clean culture pathogen tuberculosis from bodies animal, infected researched material, a same for definitions virulence of mycobacteria. The test material is treated with sulfuric acid to release from outsider microflora, neutralize and introduce subcutaneously nautical mumps and rabbit with negative tuberculin reactions. Through 4 months, if the animal will not die, it is slaughtered, macro- and microscopic examination is carried out bodies and do crops. M.tuberculosis highly pathogenic for maritime pigs and few pathogenic for rabbits. M. bovis is highly pathogenic for rabbits.

IX. SERODIAGNOSIS

use in quality additional text RSK and RPGA. Positive results are celebrated at active

tuberculosis, a same at infection mycobacteria tuberculosis and vaccination

X. SKIN-ALLERGIC TRY

Put with tuberculin (RPO) - purified protein fraction obtained from mycobacteria tuberculosis, for characteristics, estimates currents tuberculosis process, definitions efficiency vaccination and selection contingents for revaccination vstuberculosis. Tuberculin introduce intradermally in strictly certain dosage (reaction Mantu). The result is taken into account after 24-48 hours by the formation of hyperemia and papules

SPECIFIC PREVENTION
Vaccine BCG. live, freeze-dried dried culture apathogenic strain mycobacteria tuberculosis, received French scientists A) Calmette and M. Guerin. Applies intradermally for active specific prevention tuberculosis.

TIMELINE

- | | |
|--|-------------------|
| 1. Definition original level knowledge | ----- thirty min. |
| 2. Independent Job | ----- thirty min. |
| 3. Examination protocols | ----- 10 min. |
| four. Cleaning working places | ----- 10 min. |
| five. Control final level of knowledge and exercise on house | ----- 10 minutes |

PRACTICE #13 THEME:

DIAGNOSTICS OF SYPHILIS

Educational goal:

1. Train students methods microbiological diagnostics and specific prevention syphilis.

PLAN:

5. Taxonomy and main biological properties causative agents of syphilis.
6. Epidemiology, pathogenesis, immunity syphilis.
7. Principles microbiological syphilis diagnosis,
8. Preparations for etiotropic therapy and specific prevention syphilis.

INDEPENDENT JOB

1. staging reactions binding complement Wasserman.

EQUIPMENT

1. Demonstration: RSK with syphilis
2. Demonstration: micropreparations spirochetes.
3. Tank. drugs
4. tables

METHODOLOGICAL RECOMMENDATIONS

Syphilis - chronic systemic venereal infectious disease With defeat skin, mucous membranes, internal organs, bones, nervous systems With successive stages of disease caused by bacteria species *Treponema pallidum* (pale treponema) subspecies *pallidum* , related to kind treponema (*Treponema*) families Spirochaetaceae .

Etiology. Syphilis is transmitted mainly through sexual contact, and therefore refers to a group of sexually transmitted diseases, or STIs (sexually transmitted infections). However possible broadcast syphilis and through blood, for example, at transfusion blood syphilis-infected donor, or injection drug addicts when using common syringes and / or common containers for drug solutions, or in everyday life when using general "bloody" tool type dental brushes or razors.

The household "bloodless" route of infection with syphilis is also not excluded, but it is very rare and requires close contact With sick tertiary syphilis having open syphilitic ulcers or decaying syphilitic gummas, of which the causative agent can get, for example, on the dishes from which the patient drank. You can also list towels, spoons, toothbrushes, linen, etc. in contact with mucous membranes items. The ability of the patient's urine and sweat to transmit infection has not been proven, in saliva pale treponema are found only at availability rashes in cavities mouth.

It is possible that the infection of the child with mother's milk, even in the absence of visible changes in area of the breast, semen is also contagious, even in the absence of visible pathological foci on the patient's penis. Medical personnel may become infected disease at implementation medical and diagnostic events, a same at autopsy of the corpses of patients with syphilis, the corpses of children with primary congenital form diseases.

Pathogenesis. Incubation period primary stages syphilis is in average 3 weeks (interval from several days to 6 weeks) from the moment of infection. At the end incubation period in case of sexual or domestic infection at the site of penetration microbe usually develops primary affect.

Pathogenesis syphilis conditioned reaction organism on implementation in organism patient with pale treponema. The features of the pathogen determine the polymorphism leaking in infected body processes, in dependencies from stages diseases pathological changes different enough much.

AT classical flow syphilitic infections accepted allocate four period:

- Incubation;
- Primary;
- Secondary;
- Tertiary.

Latest three period are found characteristic symptoms incubation period does not manifest itself in any way, and its terms are determined only indirectly after the appearance clinics.

Diagnostics. Diagnosis syphilis in some cases can suspect clinically, but main method diagnostics and confirmation preliminary diagnosis is serodiagnosis. Currently, to determine the antibodies to the pathogen is used ELISA, earlier in Russia the Wasserman reaction was used for this. All diagnostic methods syphilis are separated on the following groups:

- Direct and indirect (indirect)
- Treponemal (specific) and non-treponemal (non-specific)
- Qualifiers (screening) and confirming (diagnostic)
- instrumentation, instrumentless.

Direct treponemal methods diagnostics allow discover pathogen directly in the biomaterial. These methods are dark-field microscopy, infection syphilis rabbits, cultural methods, PCR diagnostics.

Each of these methods has its own specific disadvantages that limit its mass application. Dark field microscopy can detect the pathogen only at fresh syphilis and With his help impossible estimate dynamics and treatment effectiveness. Method of infection with syphilis in rabbits is expensive and slow, and also does not allow to assess the patient's condition in dynamics. cultivation pale treponema on artificial environments extremely difficult, in connections With sensitivity pathogen to conditions environment. Method PCR diagnostics allows effectively detect the pathogen only in primary and secondary syphilis, test systems are relatively expensive, and studies of the effectiveness of this method in diagnosing syphilis is still going on. Thus, we see that direct diagnostic methods hardly applicable in clinical practice, and therefore, the basis of diagnosis are various serological methods (indirect).

In accordance with the current order of the Ministry of Health of the Russian Federation No. 87 dated March 26, 2001 "On improving serological diagnostics syphilis" at gray and liquor diagnostics syphilis allowed usage next reactions.

- Microreactions precipitation (indirect screening method)

- Reactions passive indirect agglutination (RPGA)
- Reactions immunofluorescence (RIF)
- Reactions immobilization pale treponem (RIBT)
- Enzyme immunoassay does not require separate regulation due to with what, in Order No. 87 not indicated.

It should be noted that none of the diagnostic methods guarantees 100% detection. pathogen. Sensitivity methods is 90-98%, so simultaneous usage 2 various methods research maybe With very high degree credibility install loyal diagnosis.

TIMELINE

- | | |
|--|-------------------|
| 1. Definition original level knowledge | ----- thirty min. |
| 2. Independent Job | ----- thirty min. |
| 3. Checking Protocols | ----- 10 min. |
| four. Cleaning working places | ----- 10 min. |
| five. Control final level of knowledge and task on house | ----- 10 minutes |

PRACTICAL OCCUPATION #14

DELIVERY OF THE MODULE ON THE TOPIC: DIAGNOSTICS OF DIPHTHERIA, PERTUSSIS, TB, leprosy, SYPHILIS

PRACTICE #15 THEME: SARS

DIAGNOSIS And FLU

Educational goal:

1. Train students methods virological diagnostics and specific prevention flu, SARS.

PLAN:

2. Taxonomy and main biological properties pathogens flu, SARS,
2. Epidemiology, pathogenesis, immunity called diseases.
3. Principles microbiological diagnostics flu, SARS
4. Preparations for etiotropic therapy and specific prevention flu, SARS, measles, rubella, wind smallpox, epidemic mumps.

INDEPENDENT JOB

1. Parsing supplies and accounting results REEF with SARS (demonstration).
2. Parsing the delivery and record keeping RTGA for seroidentification in case offlu (demonstration).
3. Parsing supplies and accounting results ELISA for serodiagnosis at SARS (demonstration).

EQUIPMENT

1. accounting results REEF at SARS (demonstration).
2. accounting results RTGA for seroidentification at flu

(demonstration).

3. accounting for ELISA results for serodiagnosis in SARS (demonstration).

METHODOLOGICAL RECOMMENDATIONS

Flu (from fr. *grippe*) — acute infectious disease respiratory ways, caused by the influenza virus. Included in the group of acute respiratory viral infections (ARVI). Periodically spreads in the form of epidemics and pandemics. Currently more than 2000 variants of the influenza virus have been identified, differing in antigenic spectrum.

The virus was first isolated in the 1930s. Influenza viruses belong to the family *Orthomyxoviridae*, which includes the genera Influenza A, B, C. Antigenic properties of internal proteins virion (M1 and NP) define belonging flu virus to kind AND, AT or FROM.

epidemic meaning for people have viruses, containing three subtype HA(H1, H2, H3) and two NA subtypes (N1, N2). Influenza A and B viruses contain NA and HA as major structural and antigenic components viral particle, possessing hemagglutinating and neuraminidase activities. At virus influenza FROM No neuraminidase, he has instead of this hemagglutinin-esterase (penetratinD) protein (HEF). A thread RNA surrounded protein and packed in lipoprotein membrane. Virions able agglutinate erythrocytes and elute in them With help virus-specific enzymes.

The influenza virus has a spherical shape with a diameter of 80-120 nm, in the center are RNA fragments, prisoners in lipoprotein shell, on surfaces which there are spikes made of hemagglutinin (H) and from neuraminidase (N). Antibodies, produced in answer on hemagglutinin (H) constitute basis immunity vs certain subtype pathogen influenza

source infections is sick human With explicit or erased form disease that releases the virus with coughing, sneezing, etc. The patient is contagious from the first hours diseases and before 5th–7th days disease.[5] Characterized aerosol (inhalation tiny drops of saliva, mucus that contain the influenza virus) transmission mechanism and extremely rapid spread in the form of epidemics and pandemics. influenza epidemics, caused serotype AND, arise approximately every 2-3 of the year, a caused serotype AT

- every 4-6 years. Serotype C does not cause epidemics, only sporadic outbreaks in children and weakened people. AT form epidemics meets more often in autumn-winter period. The periodicity of epidemics is associated with frequent changes in the antigenic structure of the virus during stay his in natural conditions.

Input gate for virus influenza are cells flickering epithelium upper respiratory tract - nose, trachea, bronchi. The virus replicates in these cells leads to their destruction and death. This explains the irritation of the upper respiratory ways of coughing, sneezing, nasal congestion. Penetrating into the blood and causing viremia, the virus renders direct, toxic action, emerging in form raise temperature, chills myalgia, headache. Besides, virus increases vascularity permeability, causes development stasis and plasma hemorrhage.

Vaccination is the traditional way to prevent influenza. Proposed vaccine for the prevention of influenza in the form of live, killed (inactivated), subunit vaccine. Vaccination is especially indicated in risk groups - children, the elderly people with chronic heart and lung diseases, and also doctors. Usually carried out, when epidemiological forecast testifies about expediency mass events (usually in the middle of autumn). A second inoculation in the middle is also possible winters.

For rapid diagnosis of influenza use "express method" of virus detection influenza With help fluorescent antibodies. researched material take from nose in first days illness. cooked from him smears handle specific flu-like fluorescent sera. formed complex antigen- the antibody glows brightly in the nucleus and cytoplasm of cells of the cylindrical epithelium and clearly visible in luminescent microscope. Answer can receive through 2-3 h.

Serological research help retrospective diagnostics flu. Examine paired blood sera taken from patients in the acute period of the disease (up to the 5th day from the onset of the disease) and during the period of convalescence with an interval of 12-14 days. Most indicative in serological diagnosis are the complement fixation reaction (RSK) With flu-like antigens and reaction braking

hemagglutination (RTGA). Diagnostic counts titer increase antibodies in four times and more.

TIMELINE

- | | | |
|--|-------|-------------|
| 1. Definition original level knowledge | ----- | thirty min. |
| 2. Independent Job | ----- | thirty min. |
| 3. Examination protocols | ----- | 10 min. |
| four. Cleaning working places | ----- | 10 min. |
| five. Control final level of knowledge and exercise on house | ----- | 10 minutes |

PRACTICAL OCCUPATION #16

THEME: DIAGNOSTICS PARENTERAL HEPATITIS, HERPES And HIVINFECTIONS

Educational goal:

1. To train students in the methods of virological diagnostics and specific prevention hepatitis B, C, D, G, HIV infections.

PLAN:

5. Taxonomy and main biological properties pathogens hepatitis B, C, D, G, HIV infection, herpes
Epidemiology, pathogenesis, immunity called diseases.
6. Principles microbiological diagnostics hepatitis B, C, D, G, HIV infection, herpes.
7. Preparations for etiotropic therapy and specific prevention hepatitis B, C, D, G, HIV-infections, herpes.
8. Change module.

INDEPENDENT JOB

1. Analysis of the formulation and accounting of results ELISA reactions for serodiagnosis and seroidentification for hepatitis B, C, D, G, HIV infections (demonstration).
2. Analysis of the formulation and accounting of results RPHA reactions in hepatitis B (demonstration).
3. Parsing staging and accounting results RTGA and ELISA for serodiagnosis (demonstration).

EQUIPMENT

1. Accounting results reactions ELISA for serodiagnosis and seroidentification for hepatitis B, C, D, G, HIV infections (demonstration).
2. Accounting for results RPHA reactions in hepatitis B (demonstration).
3. Accounting for results RTGA and ELISA for serodiagnosis (demonstration).

METHODOLOGICAL RECOMMENDATIONS

Hepatitis B is a viral disease caused by the hepatitis B virus in the literature, it may be referred to as "HBV virus", HBV or HBV) from the family hepadnaviruses.

Virus is different extremely high sustainability to various physical and chemical factors: low and high temperatures (in volume including boiling), repeated freezing and thawing, prolonged exposure to an acidic environment. In external environment at room temperature virus hepatitis A AT maybe persist before several weeks: even in a dried and imperceptible stain of blood, on a razor blade, the end needles. In the blood serum at temperature of +30°C, the infectivity of the virus remains in flow 6 months at -20°C about 15 years old. Inactivated at autoclaving in flow thirty minutes sterilization dry heat at temperature 160°C in flow 60 minutes warming up at 60°C in flow 10 hours.

The mechanism of infection transmission is parenteral. Infection occurs naturally (sexual, vertical, domestic) and artificial (parenteral) ways. Virus present in blood and various biological liquids — saliva urine, semen, vaginal secret, menstrual blood and others contagiousness (infectiousness) virus hepatitis B exceeds contagiousness HIV in one hundred once.

Greatest meaning earlier everywhere had exactly parenteral way — infection at medical and diagnostic manipulation, accompanied violation integrity skin or mucous cover through medical, dental, manicure and other tools, transfusion blood and her drugs.

Pathogenesis. Most significant pathogenetic factor at viral hepatitis AT — death infected hepatocytes due to attacks own immune agents. massive death hepatocytes leads to violation functions liver, before Total detox, in lesser degree — synthetic.

Incubation period (time from infection to onset of symptoms) hepatitis AB amounts to average 12 weeks but maybe hesitate ranging from 2 before 6 months. The infectious process begins from the moment the virus enters the bloodstream. After hit viruses in liver through blood goes hidden phase breeding and accumulation viral particles. When a certain concentration of the virus in the liver is reached, acute hepatitis C. Sometimes spicy hepatitis passes for human practically imperceptibly, and is detected by chance, sometimes proceeds in a mild anicteric form - it manifests itself only malaise and decreased performance. Some researchers believe what asymptomatic flow, anicteric the form and "icteric" hepatitis constitute equal in the number of affected individuals in the group. That there are identified diagnosed cases acute hepatitis B are only one one third of all cases acute hepatitis.

Vaccination. Mandatory vaccination. Recently, vaccination against hepatitis AT was enabled in mandatory the calendar vaccination. newborns most sensitive to virus hepatitis A AT - in case infections in this age, risk acquisition of a chronic form of hepatitis B is 100%. At the same time, immunity created by the vaccine during this period of life, the most persistent. Recommended to vaccinate newborn more in maternity house, then through 1 month after first vaccinations, and through 6 months after the first vaccination (so-called 0-1-6 regimen). When you miss another injections should be remembered about the allowable intervals - 0-1(4)-6(4-18) months. However, if allowable intervals have been missed, it is necessary to continue vaccination according to the scheme, as if there were no passes. If vaccination was carried out according to the standard scheme, repeated vaccination usually not required, since immunity preserved on lesser least in for 15 years. To determine how long immunity lasts during life, further research is needed - after all, vaccination began to be used relatively recently. Only after holding Total course vaccination, achieved almost 100% immunity. About five% general populations not answers on vaccination, in these cases should use other kinds vaccines vs hepatitis A C.

laboratory diagnostics GW - founded on identifying specific for GW antigens and relevant antibodies in blood, a same viral nucleic acids, main from which are:

HB sAg - anti-HB s
anti-HBc class Ig M and IgG
HBe Ag - anti-HWe
DNA HBV

The most widely used in the diagnosis of HB is the determination of HBsAg. This antigen is detected in both acute and chronic disease (however, acute infection usually confirmed presence high credits anti-HBs IgM). At acute GW the surface antigen of the virus is detected after 3-5 weeks from the moment of infection, that is, long before the appearance of clinical signs of the disease and in these cases is the only serological marker. HBsAg is constantly detected in preicteric and icteric periods illness. persistence HBsAg in flow 6 months and more indicates on protracted or chronic

flow disease, and allows suppose chronic carriage of the virus. Elimination of HBsAg and the appearance of antibodies to it is indispensable condition convalescence. Serological markers replication HBV are - anti-HBc class IgM, HBeAg, DNA and DNA polymerase, which are detected at acute GW With first days clinical manifestations and may show up at exacerbation of chronic hepatitis B. Serological markers of HBV replication are defined as purposes general diagnostics, So and for estimates efficiency applied therapy.

Hepatitis D virus (HDV) was first discovered in 1977. He does not belong to one of the known families of viruses. NDV is a spherical particle, in center which is spherical antigen (ND-AD), containing RNA. Outdoor the shell of the particle is formed by the surface antigen of the hepatitis B virus - HBs antigen (HBsAD). HDV cannot exist without the replication of the HB virus, which is why it is called virus - parasite or defective virus. Virus hepatitis A AT performs at this helper function, then eat role assistant for breeding NDV. Therefore, NDV - infection always occurs together with HBV infection. NDV is located mainly in nuclei hepatocytes and occasionally in cytoplasm.

Epidemiology. NDV- infection wide common. Intensity circulation NDV in different regions of the world varies significantly, but in general repeats the situation with HBV, although not completely accurate. In acute hepatitis, antibodies to HDV are secreted into different regions in 2-7% of patients, and in chronic hepatitis - in 9-50% of patients. On territories former the USSR among "healthy" carriers HBsAg greatest frequency (10-20

%) detection of antibodies to HDV detected in Moldova, Kazakhstan, Central Asia, Tuva, then found in areas hyperendemic for HBV. In the European part of Russia, the frequency of detection antibodies to NDV is 1.2-5.5 %.

The source of infection are patients with acute and chronic IOP, virus carriers, and also carriers of anti-HDV, since it is known that in individuals with anti-HDV, it is possible to simultaneously detect RNA-NDV. Transmission of HDV occurs in the same way as with HBV (parenteral, sexually, from mother to fetus). K delta - infections are susceptible to persons who have not been ill HBV (i.e. not having anti-HBs), as well as carriers of the HB virus (healthy carriers of HBsAg and patients with chronic HBV). Delta infection occurs both sporadically and outbreaks.

Pathogenesis, clinic. The infectious process caused by HDV manifests itself before Total appearance ND-Ag in blood. Delta - antihemia maybe to be short-term or lengthy depending on how it happened infection and whether integration of the HB virus into the hepatocyte genome. Distinguish between acute, protracted and chronic course of delta infection. The nature of its flow is limited by the duration of HBs- antigenemia: by as it is depleted, the synthesis of HDV also stops, and delta- dependent pathological process.

Delta infection develops as a coinfection or superinfection. With co-infection going on simultaneous infection HBV + NDV at persons, not ill previously HBV - infection (not having HBV infection markers before infection). In this case develops acute HBV + HD-hepatitis with the appearance of serological markers of two acute infections. At co-infections replication HBV more often Total HBV + IOP - hepatitis A usually it happens sharp and ends recovery.

At superinfection NDV - infection stratified on current HBV- infection at healthy carriers of HBsAg, in convalescents of the main HBV, in patients with chronic HBV. At the same time, a clinic of acute viral hepatitis delta develops, accompanied by appearance antibodies to delta antigen.

Laboratory diagnosis of hepatitis D (HD) The hepatitis D virus (HDV) is a defective a single-stranded RNA virus that needs help to replicate HBV for the synthesis of envelope proteins consisting of HBsAg, which is used for encapsulation genome IOP. IOP not belongs neither to alone from famous families animal viruses, in terms of its properties, IOP is closest to viroids and satellite viruses plants. laboratory diagnostics carried out through detection serological markers IOP, including Availability antigen, antibodies to him and HDR RNA. Detection antigen IOP and RNA IOP in serum blood or fabrics liver testifies about availability active HD infections, but, should Mark, what these markers may not show up in serum of patients with fulminant GD. Marker active replication IOP is also an anti-HDP IgM class. Serological markers HD infections depend on how the virus was acquired - in the form of co-infection with HBV (in most patients the disease has an acute course and ends with recovery) or superinfection at sick With chronic hepatitis B infection

(flows heavier, how co-infection - 10% develop fulminant hepatitis). During superinfection in patients with chronic hepatitis B infection serological painting It has the following characteristic features: - the HBsAg titer decreases by the time the IOP antigen appears in the serum; - antigen IOP and RNA-HVD continue determined in serum So How usually at most patients with HD superinfection (70-80%) chronic infection develops, in contrast to cases of coinfection; - high titers of antibodies (anti-VOP) are determined as class IgM, So and IgG, which persist indefinite time. Serological markers of the HD virus are determined by enzyme immunoassay and radioimmunoassay, and RNA-HVD - method polymerase chain reactions.

Hepatitis C — anthroponotic viral disease With parenteral mechanism infection, most often flowing in form post-transfusion hepatitis A With predominance anicteric and prone to chonrization.

Hepatitis FROM called "affectionate killer" due to capabilities mask true reason under the guise sets others diseases.

Parenteral viral hepatitis C is caused by an RNA virus with a size virion 30-60 nm, belonging to the Flaviviridae family. HCV virus particles have membrane, contained in the blood in trace amounts and associated with lipoproteins low density and antibodies to proteins virus hepatitis A FROM. viruses, dedicated from complexes With lipoproteins and anti-HCV antibodies have diameter 60-70 nm. At electron microscopic studying on surfaces virion identified Good pronounced ledges tall 6-8 nm.

source infections are sick With active hepatitis C and latent sick — carriers virus. HCV infection is infection With parenteral mechanism infection - through infected blood and its components. Infection is possible with parenteral manipulations, including in medical institutions, including the provision of dental services, through injection equipment, acupuncture, piercing, drawing tattoos, when rendering range of services in hairdressing, but at genital contacts, the likelihood of getting hepatitis C is much less than hepatitis B, and boils down to minimal indicators.

laboratory diagnostics hepatitis C (GS). laboratory diagnostics HS was solved using modern methods of molecular biology, given that in HS the virus is in an extremely low concentration and its antigens are not available for detection with help contemporary methods indication, efforts researchers concentrated on detection of antibodies to various antigenic components of the virus, the detection of which can serve as an indicator of the presence of the virus. Proteins were used as antigens. coded structural and non-structural zone HCV RNA, received at help recombinant technology or synthesis (polypeptides, used in contemporary immunological methods - C22-3; C33s, C100-3, C200, NS5, S-1-1). laboratory diagnostics HS based on discovery serological markv HCV: antibodies to virus HS (anti-HCV, anti-HCV class IgM, IgD) method ELISA and HCV RNA method PCR. To date, 4 generations of test systems have been developed for the detection of anti-HCV in enzyme immunoassay, but the first generation ELISA is not currently used due to low sensitivity. HCV RNA is indicator active replication HCV and most early marker infection, and maybe to be discovered method polymerase chain reactions already through 1- 2 weeks after infection, shortly before raise level serum transaminases. Anti-HCV are found to 5-6 week after start hepatitis A in 80% cases and to 12 week at 90% persons method enzyme immunoassay analysis. At in the determination of anti-HCV, in some cases a false positive reaction is recorded. To distinguish between false positives and true samples antibodies to HCV developed additional tests - recombinant immunoblotting, spectrum determination proteins anti-HCV.

HIV — virus immunodeficiency human, defiant disease — HIV infection last stage which known How syndrome acquired immunodeficiency (AIDS) — in difference from congenital immunodeficiency.

The spread of HIV infection is associated mainly with unprotected sex. contacts, use of virus-contaminated syringes, needles and other medical and paramedical instruments, transmission of the virus from an infected mother to her child during during childbirth or while breastfeeding. Mandatory testing in developed countries donated blood has greatly reduced the possibility of transmission of the virus during its use.

HIV primarily infects cells of the immune system (CD4+ T-lymphocytes, macrophages and

dendritic cells), as well as some other types of cells. HIV-infected CD4+ T-lymphocytes gradually are dying.

The human immunodeficiency virus belongs to the family of retroviruses (Retroviridae), genus lentiviruses (Lentivirus). Name Lentivirus going on from Latin words lente — slow. Such title reflects one from features viruses this groups, a exactly — slow and unequal speed development infectious process in macroorganism. For lentiviruses same typical long incubation period.

Diagnostics. Flow HIV infections characterized lengthy absence significant symptoms disease[81]. Diagnosis HIV infections put on basis laboratory data: when detecting antibodies to HIV in the blood. Antibodies to HIV during the period the acute phase, as a rule, is not detected. In the first 3 months after infection with antibodies HIV is detected in 96-97% of patients, after 6 months. - the rest have 2-3%, and in later terms - only 0.5-1% (source Centers for Disease Control and Prevention USA, 2009). AT stages of AIDS register a significant decrease in the content of antibodies in the blood. First weeks post-infection represent the “seronegative window period” when antibodies to HIV are not detected. Therefore, a negative HIV test result in this period not means what human not infected HIV and not maybe infect others.

For the diagnosis of lesions of the oral mucosa in HIV-infected patients accepted working classification, approved in London, in September 1992 of the year. Everybody defeat divided on 3 groups:

1 group — defeat, clearly related With HIV infection. AT this group included the following nosological forms:

candidiasis (erythematous, pseudomembranous, hyperplastic, atrophic); hairy leukoplakia;
marginal gingivitis;
ulcerative necrotic gingivitis;
destructive periodontitis; sarcoma
Kaposi;
non-Hodgkin lymphoma.

2 group - lesions less clearly associated with HIV infection: bacterial
infections;
diseases of the salivary glands;
viral infections; thrombocytopenic
purpura.

3 group — defeats, which may to be with HIV infection, but not related With her.

Herpes (Greek ἑρπης - creeping, spreading skin disease) - viral disease with a characteristic eruption of grouped vesicles on the skin and mucous membranes shells.

Simple herpes (Herpes simplex) — group crowded bubbles With transparent contents on an inflamed base. Herpes is preceded by itching, burning of the skin, sometimes chills, malaise.

Shingles lichen (Herpes zoster) — characterized pain on move nerve, head pain. Through some days on site skin on move nerve appear rashes in the form of grouped vesicles, first with a transparent, and later purulent bloody content. Enlarged lymph nodes, fever body, the general condition is disturbed. Neuralgic pains can last up to several months.

Pathogenesis. The herpes virus is transmitted by direct contact, as well as through household items. It can also be transmitted by airborne droplets way. Herpes penetrates through the mucous membranes of the oral cavity, upper respiratory tract and genitals. Having overcome tissue barriers, the virus enters the blood and lymph. Then hits in various internal organs.

The virus penetrates the sensitive nerve endings and integrates into the genetic apparatus nervous cells. After this delete virus from organism impossible, he will remain With human on all life. immune system reacts on penetration herpes development specific antibodies, blocking circulating in blood viral particles. Characteristically awakening infections in cold time of the year, at colds diseases, at hypovitaminosis. reproduction herpes in cells epithelium skin and mucous shells leads to development of dystrophy and death cells.

According to research scientists Colombian University, herpes is stimulating factor for the development of Alzheimer's disease. Later, these data were independently validated by researchers

at the University of Manchester. Previously the same a group of researchers led by Ruth Itzhaki proved that the herpes simplex virus found in the brains of nearly 70% of patients with Alzheimer's disease. In addition, they confirmed what at infection virus culture cells brain going on a significant increase in the level of beta-amyloid, from which plaques are formed. During recent study, scientists were able to find that 90% of the plaques in the brains of patients with sickness Alzheimer contain DNA simple herpes — HSV-1.

For diagnostics herpetic infections are used Everybody laboratory reactions — from cytological research before molecular biological methods.

Material for virus isolation for the diagnosis of herpes infection maybe serve content herpetic bubbles, scrapings With horny shells and liquids from front cameras eyes, blood, saliva, urine, spinal liquid feces pieces of tissue of the brain, liver, kidneys, spleen, lungs, lymph nodes taken for bio- or autopsy.

Infectious material can for a long time keep at -70°C , then How at temperature -20°C he quickly is inactivated. Virus containing fabrics may to be saved more 6 months at 4°C , if they are situated in 50% solution glycerin.

Exist whole row special methods for identifying viral antigens, specific antibodies and virus-induced morphologically changed cells.

Most accessible and technically uncomplicated is cytological method, allowing explore morphological changes in cells virus-infected simple herpes. The effectiveness of the method depends from receiving sufficient quantities cells for research. Availability intranuclear inclusions, characteristic for reproductions virus herpes, serves confirmation diagnosis. Should remember, what intranuclear inclusion are found only after immediate commits smears scraping in absolute alcohol With subsequent coloration on Romanovsky-Giemsa. Morphological changes, induced virus simple herpes, can same discover in sections fabrics infected organs. characteristic for herpetic infections is: Availability multi-core cells, intranuclear inclusions and in some cases of hemorrhage. With a generalized form of the disease multi-core cells With eosinophilic inclusions find in zones necrotic fabrics various bodies (brain, liver, kidney, adrenal glands, epithelium bronchi and trachea).

Method immunofluorescence — is method express diagnostics herpetic infections and allows in flow 1-2 hours determine Availability herpesvirus antigens in the clinical material (scraping from the skin and mucous membranes, sections of biopsied organs). Identification of herpes simplex virus antigens to be completed in various modifications method immunofluorescence — straight, indirect, With application labeled complement.

From serological methods identification most often use reaction binding complement (RSK), especially in micromodifications her staging. micromethods use and for identifying virus simple herpes in reactions neutralization, passive hemagglutination and in others serological tests. Sensitivity listed methods are different.

AT the present time one from most sensitive methods diagnostics herpes infection is an enzyme immunoassay (ELISA) method that allows detect, depending on the type of biological material, how virus-specific antigens, so virus-specific antibodies class IgM, IgG.

TIMELINE

1. Definition original level knowledge ----- 30 min.
2. Independent job ----- 30 min.
3. Examination protocols ----- 10 min.
4. Cleaning workplace ----- 10 min.
5. Control final level knowledge and task on house ----- 10 min.

PRACTICAL OCCUPATION #17

CHANGE MODULE ON TOPIC: "VIRUS - ACTUATORS INFECTION

TEST TASKS

***ON MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY FOR
MEDICAL FACULTY***

Vladikavkaz

«GENERAL MICROBIOLOGY. STRUCTURE OF A BACTERIAL CELL»

I OPTION

(Choose one or some correct answers)

1. Essence scientific discoveries D.I. Ivanovsky:

- A) the creation of the first microscope;
- B) opening viruses;
- C) opening phenomena phagocytosis;
- D) receiving anti-rabies vaccines.

2. Main morphological varieties bacteria:

- A) cocci;
- B) Sticks;
- C) Vintage;
- D) Branching.

3. prokaryotes, not having cellular walls and not synthesizing predecessorspeptidoglycan:

- A) Staphylococci;
- B) Neisseria;
- C) streptococci;
- D) Mycoplasmas.

4. To spore-forming bacteria relate:

- A) streptococci;B)
- Clostridia;
- C) Neisseria;
- D) Salmonella.

5. What properties have spirochetes?

- A) have thin cellular wall;
- B) gram-negative;
- C) Thin spirally curved cells;
- D) Have cytoplasmic cylinder

6. Chlamydia:

- A) Gram-negative;
- B) form disputes;
- C) Prokaryotes;
- D) obligate intracellular parasites.

7. Properties LPS:

- A) It is an endotoxin;
- B) Thermolabile;
- C) Is O antigen;
- D) Contains peptidoglycan.

8. Outdoor membrane Gram-negative bacteria It has:

- A) LPS;
- B) Porins;
- C) Lipid AND;
- D) Peptidoglycan.

9. Gram positive bacteria:

- A) Escherichia;
- B) Staphylococci;
- C) Vibrios;
- D) Streptococci.

10. Lasting slimy layer, located outside cellular walls bacteria:

- A) Case;
- B) Mucoid;
- C) Outer membrane;
- D) Capsule.

11. Non-spore-forming acid resistant bacteria:

- A) Clostridia
- B) Escherichia;
- C) bacilli;
- D) Mycobacteria.

12. Chromosomes bacteria:

- A) Associated with the cytoplasmic membrane;
- B) Contain histones;
- C) have the shape of a ring;
- D) Connected with LPS.

13. Darkfield microscopy applied for study:

- A) Escherichia coli;
- B) Rickettsia;
- C) Staphylococcus;
- D) pale treponema.

14. name method coloring, applicable for pathogens tuberculosis:

- A) Ziel-Nielsen;
- B) Ozheshko;
- C) Burri-Gins;
- D) Neisser.

15. Methods definitions availability flagella bacteria :

- A) Etching and impregnation salts silver or mercury;
- B) Coloring on Neisser;
- C) Coloring on Leffler;
- D) By directed character movements at bacteria in preparations "crushed" and "hanging drop."

16. What such transformation?

- A) Broadcast genetic information at contact bacterial cells different "sexual" orientation;
- B) Recovery damaged DNA;
- C) The transfer of genetic material through highly polymerized DNA.

17. signs mushrooms:

- A) The main component of the cell wall is chitin;
- B) Have chlorophyll;
- C) Contain ergosterols in cytoplasmic membrane;
- D) It has nucleus with nuclear shell.

18. protozoa, having apical complex:

- A) Balantidia;
- B) Malarial Plasmodium;
- C) Trichomonas;
- D) Toxoplasma.

19. To microorganisms with a prokaryotic type of cell organization relate:

- a) moldy mushrooms;
- b) spirochetes;
- C) chlamydia;
- D) mycoplasmas;
- E) actinomycetes.

Choose the only combination that takes into account all correct

answers:

- A) a b, in;
- B) b, in, G, d;
- C) in, G, d;
- D) a, in, G, d;
- E) b, G, d.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

- 1. Organs movements at bacteria:**
- 2. bacteria, covered flagella with all parties cells:**
 - A) drinking
 - B) Flagella;
 - C) Pseudopodia;
 - D) Trichomonas;
 - E) Peritrichs.

21.

- 1. microorganisms, not having cellular walls:**
- 2. Adhesion bacteria to eukaryotic cells:**
 - A) amphitriches;
 - B) Spirochetes;
 - C) Mycoplasmas;
 - D) Porins;
 - E) Drank;
 - F) flagella.

"GENERAL MICROBIOLOGY. STRUCTURE OF BACTERIAL CELLS»

II OPTION

(Choose one or some correct answers)

1. founding scientists physiological period development microbiology:

- A) Leeuwenhoek;
- B) Pasteur;
- C) Mechnikov;
- D) Koch.

2. Structural features of prokaryotes:

- A) ribosome sedimentation constant 70S;
- B) Available nucleoid;
- C) there is no Golgi apparatus;
- D) Absent nuclear membrane.

3. Not have complete cellular walls:

- A) Chlamydia;
- B) L-shape;
- C) Rickettsia;
- D) Mycoplasmas.

4. What morphology have sarcinas?

- A) rod-shaped;
- B) coccoid;
- C) twisted;
- D) filiform.

5. To spirochetes relate:

- A) Treponema;
- B) Borrelia;

- C) Leptospira;
- D) Mycoplasmas.

6. controversy actinomycetes participate in:

- A) reproduction;
- B) Protection from unfavorable external impacts;
- C) Settlement of the microbe or colonization of the substrate;
- D) Transfer genes.

7. Rickettsia:

- A) obligate intracellular parasites;B)
- Prokaryotes;
- C) Gram-negative;
- D) are painted on method Zdrodovsky.

8. Functions of the bacterial cell wall: A)

- Participation in the process of cell division;B) Participation in exchange substances;
- C) Protection from actions external harmful factors;
- D) Maintaining a constant forms.

9. Components LPS bacteria:

- A) Lipid AND;
- B) Peptidoglycan;
- B) polysaccharide side chain;
- D) Porin protein.

10. Microcapsule:

- A) Formed in most bacteria;
- B) Good visible in light microscope;
- C) Thickness less than 0.2 μm ;
- D) Attaches bacteria acid resistance.

11. Sustainability non-spore-forming bacteria to acids alkalis and alcoholsconditioned high content in cellular wall:

- A) Peptidoglycan;
- B) Teikhoevs acids;
- C) Peptide bridges;
- D) Voskov and lipids.

12. Peculiarities grains volutin?

- A) Relate to cytoplasmic inclusions;
- B) Are stained on Neisser;
- C) Differ in methochromasia;
- D) Contain polyphosphates.

13. Tinctorial properties bacteria characterize:

- A) Sustainability in external environment
- B) Resistance to physical factors
- C) Sensitivity to bacteriophages
- D) Attitude to certain method staining

14. Methods staining rickettsia:

- A) Coloring according to Romanovsky-Giemsa;
- B) coloring by Neisser;
- C) Coloring on Zdrodovsky;D)
- Coloring on Aueske.

15. For detection dispute at bacteria use coloration:

- A) By Neisser;
- B) According to

- Romanovsky-Giemsa;
- C) According to Burri-Gins;
- D) By Ozheshki.

16. What such conjugation?

- A) Correction damaged plots DNA;
- B) Transfer of genetic information with the help of a bacteriophage;
- C) hereditary hop change sign;
- D) Broadcast genetic information at crossing bacterium through genital villi.

17. signs mushrooms:

- A) Absent chlorophyll;
- B) Have rigid cellular wall;
- C) Contain ergosterols in cytoplasmic membrane;
- D) Eukaryotes.

18. Protozoa:

- A) eukaryotes;
- B) belong to the animal kingdom;
- C) Have cellular structure;
- D) Relate to prokaryotes.

19. Light microscopy includes the following varieties: a) phase-contrast microscopy; b) electron microscopy; c) darkfield microscopy; d) microscopy in dark field; e) immersion microscopy.

Choose the only combination that takes into account all correct answers:

- A) a, in, G, d;
- B) a, b, G, d;
- C) b, in, G, d;
- D) b, in, G;
- D) in, G, d.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

1. Functions pili (villi, fimbria):

2. Nucleoid bacteria:

- A) Adhesion bacteria to substrate;
- B) Are antigens;
- C) Serve as a receptor for bacteriophages;
- D) Contains 2-3 nucleolus;
- E) The DNA strand is closed in a ring;
- E) Has a nuclear shell.

21.

1. Collection forms bacteria:

2. spore-forming bacteria:

- A) Clostridia
- B) bacilli;
- C) Actinomycetes;
- D) Spirilla;
- E) Mycoplasmas;
- F) Spirochetes.

"GENERAL MICROBIOLOGY. STRUCTURE BACTERIAL CELLS»

II OPTION

(Choose one or some correct answers)

1. FROM name what scientist related opening entities fermentation [1857], microbial conditionality and contagiousness of infectious diseases [1881], methods manufacturing vaccines and ways prevention chicken cholera Siberian ulcers and rabies [1882-1885] ?
 - A) Leeuwenhoek;
 - B) Mechnikov;
 - C) Koch;
 - D) Pasteur.
2. **Determine concept "taxon":**
 - A) Genetically homogeneous pure culture of microbes;
 - B) culture germs, ongoing from one cells;
 - C) culture certain kind germs, dedicated from environmental environment, pathological materials of humans and animals or received from museum;
 - D) Group microorganisms, united in systematic category on basis commonality properties and signs.
3. **Eukaryotes:**
 - A) the simplest;
 - B) Eubacteria;
 - C) Mushrooms;
 - D) Prions.
4. **bacteria, at which there is no full cellular wall:**
 - A) Rickettsia;
 - B) Mycoplasmas;
 - C) Chlamydia;
 - D) Spirochetes.
5. **Curled bacteria:**
 - A) Actinomycetes;
 - B) Spirilla;
 - C) Mycobacteria;
 - D) Spirochetes.
6. **Actinomycetes:**
 - A) Gram positive microbes;
 - B) Cells have view branched threads;
 - C) form exospores;
 - D) Prokaryotes.
7. **Microorganisms, partially or completely lost cellular wall under action factors external environments:**
 - A) spheroplasts;
 - B) Protoplasts;
 - C) L-shape;
 - D) Mycoplasmas.
8. **Functions LPS:**
 - A) antigenic;
 - B) Enzymatic;
 - C) Toxic (endotoxin);
 - D) Hereditary.
9. **Microbes in which cell wall rigidity causes peptidoglycan:**
 - A) Gram-negative bacteria;
 - B) Viruses;
 - C) Gram-positive bacteria;
 - D) Mushrooms.
10. **Acid resistant microorganisms:**
 - A) Mycobacteria;
 - B) Streptococci;

- C) Vibrios;
- D) Staphylococci.

11. Functions pili (villi, fimbria):

- A) Adhesion bacteria to substrate;
- B) Participation in transfer genes;
- C) Serve receptor for bacteriophages;
- D) Are antigens.

12. Education endospore at bacteria stimulate:

- A) Flaw oxygen;
- B) Change temperature environmental environment;
- C) deficit nutritional substances;
- D) Hit in organism human or animal.

13. signs gram-positive bacteria:

- A) There are teichoic acids in the cell wall;
- B) Can form disputes;
- C) The main component of the cell wall is peptidoglycan;
- D) Individual representatives acid resistant.

14. What kind peculiarities characteristic for mesosomes at bacteria?

- A) are formed as a result of invagination of the cytoplasmic membranes in cytoplasm;
- B) Performs functions digestive vacuoles;
- C) Synthesize protein;
- D) Reveal on Ziel-Nielsen.

15. For detection capsules bacteria in clean culture usecoloring:

- A) simple;
- B) According to Neisser;
- C) By Gram;
- D) By Burri Guinsu.

16. What such transformation?

- A) Broadcast genetic information at contact bacterialcells different "sexual" orientation;
- B) Recovery damaged DNA;
- C) The transfer of genetic material throughhighly polymerized DNA.

17. higher mushrooms:

- A) have axial a thread;
- B) Have septate mycelium;
- C) Form vegetative endospores;
- D) Form exospores (conidia).

18. Give characteristic protozoa:

- A) have a cellular structure;
- B) Refer to eukaryotes;
- C) Outside surrounded by pellicle;D)
- Relate to kingdom animals.

19. To microorganisms with a prokaryotic type of cell organization include: a) mold fungi; b) spirochetes; c) chlamydia; D) mycoplasmas; e) actinomycetes.

Choose the only combination that takes into account allcorrect answers:

- A) a b, in;
- B) b, in, G, d;
- B) a in, G, d;

D) a, b, G, d;

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

1. For detection acid resistant bacteria apply?

2. For detection grains volutin bacteria?

- A) Coloring on Buri-Ginsu;
- B) Coloring on Ziel-Nielsen;
- C) Coloring on Romanovsky-Giemsa;
- D) Staining with diluted carbolic fuchsin;D) coloring by Neisser.

21.

1. Function synthesis squirrel performs:

2. chromosomal genetic structures at bacteria:

- A) mesosomes;
- B) Ribosomes;
- C) Plasmids;
- D) transposons;
- D) Nucleoid.

"GENERAL MICROBIOLOGY. STRUCTURE BACTERIAL CELLS»

II OPTION

(Choose one or some correct answers)

1. Who is one from founders immunological stagedevelopment of microbiology and the creator of the phagocytic theory immunity?

- A) Rarely;
- B) Mechnikov;
- C) Koch;
- D) Pasteur.

2. What kind microbes relate to eukaryotes?

- A) the simplest;
- B) Mycoplasmas;
- C) Mushrooms;
- D) Chlamydia.

3. Structural features of prokaryotes:

- A) ribosome sedimentation constant 70S;
- B) Available nucleoid;
- C) there is no Golgi apparatus;
- D) Absent nuclear membrane.

4. What such streptobacilli?

- A) Cocci forming a chain;
- B) sticks, generators chain;
- C) Collection forms;
- D) spore-forming sticks, conducive chain.

5. branching forms bacteria:

- A) Actinomycetes;
- B) Spirilla;
- B) Mycoplasmas;
- D) Spirochetes.

6. Microorganisms in which the absence of a cell wall is always determined genetically:

- A) protoplasts

- B) Spheroplasts
 - C) Chlamydia
 - D) Mycoplasmas
- 7. What kind properties characteristic for chlamydia?**
- A) Gram-negative;
 - B) Prokaryotes;
 - C) obligate intracellular parasites;
 - D) Have twisted shape.
- 8. Lipopolysaccharide bacterial cells situated in:**
- A) cytoplasmic membrane;
 - B) outdoor membrane gram-positive bacteria;
 - C) Mesosomes;
 - D) Outdoor membrane Gram-negative bacteria.
- 9. AT composition peptidoglycan includes:**
- A) Teichoaceae acids
 - B) N-acetylglucosamine and M-acetylmuramic acid
 - C) Lipopolysaccharide (LPS)
 - D) glycan molecules.
- 10. What kind structures required for L-shape bacteria?**
- A) a capsule
 - B) CPM;
 - B) cytoplasm;
 - D) Nucleoid;
 - D) Cellular wall.
- 11. Non-spore-forming bacteria, most sustainable to action acids, alkalis and alcohol:**
- A) Mycobacteria;
 - B) Clostridia;
 - B) Escherichia;
 - D) Bacillus.
- 12. Intracellular inclusion bacteria:**
- A) Glycogen grains;
 - B) Mitochondria;
 - C) grains of volutin;
 - D) Ribosomes.
- 13. Complex differential diagnostic methods coloring:**
- A) Tsil-Nelsen stain;
 - B) Coloring blue Leffler;
 - C) coloring by Gram;
 - D) Coloring divorced carbolic fuchsin.
- 14. On microscopy of the test material, rickettsia usually discover:**
- A) AT cytoplasmic membrane;
 - B) AT mesosomes;
 - C) extracellular;
 - D) AT cytoplasm cells.
- 15. For detection grains volutin at bacteria coloring is used:**
- A) By Neisser;
 - B) According to Romanovsky-Giemsa;
 - C) According to BurrioGuins;
 - D) By Ozheshki.
- 16. What such mutagens?**

- A) genes, providing mutation
- B) Factors defiant mutation
- C) Factors regenerating DNA;
- D) Fatory, transmitting genetic information.

17. Mycelium mushrooms - this is:

- A) Cell without cytoplasmic membranes;
- B) Aggregate hyphae;
- C) Aggregate chlamydospores;
- D) multi-core structure.**

18. Protozoa:

- A) eukaryotes;
- B) Contain a well-formed nucleus with a nuclear membrane;
- C) Arranged more difficult, how cells bacteria;
- D) Prokaryotes.

19. To microorganisms with a prokaryotic type of cell organization

include: a) chlamydia; b) viruses; c) mold fungi; D) spirochetes; e) actinomycetes; e) mycoplasmas.

Choose the only combination that takes into account all correct answers:

- A) a b, in;
- B) a, g, d, e;
- C) in, G, d;
- D) a, in, G, d;
- D) b, G, d.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

1. Transduction:

2. Conjugation:

- A) Correction damaged plots DNA;
- B) Transfer of genetic information with the help of a bacteriophage;
- C) hereditary hop change sign;
- D) Broadcast genetic information at crossing bacterium through genital villi.
- E) Transfer of genetic material using highly polymerized DNA.

21.

1. Actinomycetes:

2. Chlamydia:

- A) Gram-positive microbes;
- B) Gram negative microbes;
- C) Cells look like branched filaments;
- D) form exospores;
- D) Obligate intracellular parasites.
- E) Eukaryotes.

«GENERAL MICROBIOLOGY. STRUCTURE OF A BACTERIAL CELL»

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1. Essence scientific discoveries D.I. Ivanovsky:

- A) the creation of the first microscope;
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- B) Neisseria;
- B) streptococci;
- D) Mycoplasmas.

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- C) Is O antigen;
- D) Contains peptidoglycan.

8. Outdoor membrane Gram-negative bacteria It has:

- A) LPS;
- B) Porins;
- C) Lipid
- AND;
- D) Peptidoglycan.

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- D) Streptococci.

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- A) Case;
- B) Mucoid;

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- D) Capsule.

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- C) bacilli;
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12. Chromosomes bacteria:

- A) Associated with the cytoplasmic membrane;
- B) Contain histones;
- C) have the shape of a ring;
- D) Connected with LPS.

13. Darkfield microscopy applied for study:

- A) Escherichia coli;
- B) Rickettsia;
- C) Staphylococcus;
- D) pale treponema.

14. name method coloring, applicable for pathogens tuberculosis:

- A) Ziel-Nielsen;
- B) Ozheshko;
- B) Burri-Gins;
- D) Neisser.

15. Methods definitions availability flagella bacteria :

- A) Etching and impregnation salts silver or mercury;
- B) Coloring on Neisser;
- C) Coloring on Leffler;
- D) By directed character movements at bacteria in preparations "crushed" and "hanging drop."

16. What such transformation?

- A) Broadcast genetic information at contact bacterial cells different "sexual" orientation;
- B) Recovery damaged DNA;
- C) The transfer of genetic material through highly polymerized DNA.

17. signs mushrooms:

- A) The main component of the cell wall is chitin;
- B) Have chlorophyll;
- C) Contain ergosterols in cytoplasmic membrane;
- D) It has nucleus with nuclear shell.

18. protozoa, having apical complex:

- A) Balantidia;
- B) Malarial Plasmodium;
- C) Trichomonas;
- D) Toxoplasma.

19. To microorganisms with a prokaryotic type of cell organization relate: a) moldy mushrooms; b) spirochetes; in) chlamydia; D) mycoplasmas; e) actinomycetes.

Choose the only combination that takes into account all correct answers:

- A) a b, in;
- B) b, in, G, d;
- C) in, G, d;

D) a, in, G, d;

D) b, G, d.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

1. Organs movements at bacteria:

2. bacteria, covered flagella co all parties cells:

A) drinking

B) Flagella;

B) Pseudopodia;

D) Trichomonas;

D) Peritrichs.

21.

1. microorganisms, not having cellular walls:

2. Adhesion bacteria to eukaryotic cells:

A) amphitriches;

B) Spirochetes;

B) Mycoplasmas;

D) Porins;

D) Drank;

E) flagella.

"GENERAL MICROBIOLOGY. STRUCTURE BACTERIAL CELLS»

II OPTION

(Choose one or some correct answers)

1. founding scientists physiological period development microbiology:

A) Leeuwenhoek;B)

Pasteur;

B) Mechnikov;D) Koch.

2. Structural features of prokaryotes:

A) ribosome sedimentation constant 70S;

B) Available nucleoid;

C) there is no Golgi apparatus;

D) Absent nuclear membrane.

3. Not have complete cellular walls:

A) Chlamydia;

B) L-shape;

C) Rickettsia;

D) Mycoplasmas.

4. What morphology have sarcinas?

A) rod-shaped;

B) coccoid;

C) twisted;

D) filiform.

5. To spirochetes relate:

A) Treponema;

B) Borrelia;

B) Leptospira;

D) Mycoplasmas.

6. controversy actinomycetes participate in:

A) reproduction;

B) Protection from unfavorable external impacts;

- C) resettlement microbe or colonization substrate;
- D) Transfer genes.

7. Rickettsia:

- A) obligate intracellular parasites;
- B) Prokaryotes;
- C) Gram-negative;
- D) are painted on method Zdrodovsky.

8. Functions of the bacterial cell wall:

- A) Participation in the process of cell division;
- B) Participation in exchange substances;
- C) Protection from external harmful factors;
- D) Maintaining a constant forms.

9. Components LPS bacteria:

- A) Lipid AND;
- B) Peptidoglycan;
- B) polysaccharide side chain;
- D) Porin protein.

10. Microcapsule:

- A) Formed in most bacteria;
- B) Good visible in light microscope;
- C) Thickness less than 0.2 μm ;
- D) Attaches bacteria acid resistance.

11. Sustainability non-spore-forming bacteria to acids alkalis and alcohols conditioned high content in cellular wall:

- A) Peptidoglycan;
- B) Teikhoevs acids;
- C) Peptide bridges;
- D) Voskov and lipids.

12. Peculiarities grains volutin?

- A) Relate to cytoplasmic inclusions;
- B) Are stained on Neisser;
- C) Differ in methochromasia;
- D) Contain polyphosphates.

13. Tinctorial properties bacteria characterize:

- A) Sustainability in external environment
- B) Resistance to physical factors
- C) Sensitivity to bacteriophages
- D) Attitude to certain method staining

14. Methods staining rickettsia:

- A) Coloring according to Romanovsky-Giemsa;
- B) coloring by Neisser;
- C) Coloring on Zdrodovsky;
- D) Coloring on Aueske.

15. For detection dispute at bacteria coloring is used:

- A) By Neisser;
- B) According to Romanovsky-Giemsa;
- C) According to BurrioGuins;
- D) By Ozheshki.

16. What such conjugation?

- A) Correction damaged plots DNA;

- B) Transfer of genetic information with the help of a bacteriophage;
- C) hereditary hop change sign;
- D) Broadcast genetic information at crossing bacterium through genital villi.

17. signs mushrooms:

- A) Absent chlorophyll;
- B) Have rigid cellular wall;
- C) Contain ergosterols in cytoplasmic membrane;
- D) Eukaryotes.

18. Protozoa:

- A) eukaryotes;
- B) belong to the animal kingdom;
- C) Have cellular structure;
- D) Relate to prokaryotes.

19. Light microscopy includes the following varieties: a) phase-contrast microscopy; b) electron microscopy; c) darkfield microscopy; d) microscopy in dark field; e) immersion microscopy.

Choose the only combination that takes into account all correct answers:

- A) a, in, G, d;
- B) a, b, G, d;
- C) b, in, G, d;
- D) b, in, G;
- D) in, G, d.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

1. Functions pili (villi, fimbria):

2. Nucleoid bacteria:

- A) Adhesion bacteria to substrate;
- B) Are antigens;
- C) Serve as a receptor for bacteriophages;
- D) Contains 2-3 nucleolus;
- E) The DNA strand is closed in a ring;
- E) Has a nuclear shell.

21.

1. Collection forms bacteria:

2. spore-forming bacteria:

- A) Clostridia
- B) bacilli;
- C) Actinomycetes
- D) Spirilla;
- D) Mycoplasmas;
- E) Spirochetes.

"GENERAL MICROBIOLOGY. STRUCTURE OF BACTERIAL CELLS»

II OPTION

(Choose one or some correct answers)

- 1. FROM name what scientist related opening entities fermentation [1857], microbial conditionality and contagiousness of infectious diseases [1881], methods manufacturing vaccines and ways prevention chicken cholera Siberian ulcers and rabies [1882-1885]?**

A) Leeuwenhoek;

- B) Mechnikov;
- C) Koch;
- D) Pasteur.

2. Determine concept "taxon":

- A) Genetically homogeneous pure culture of microbes;
- B) culture germs, ongoing from one cells;
- C) culture certain kind germs, dedicated from environmental environment, pathological materials of humans and animals or received from museum;
- D) Group microorganisms, united in systematic category on basis commonality properties and signs.

3. Eukaryotes:

- A) the simplest;
- B) Eubacteria;
- C) Mushrooms;
- D) Prions.

4. bacteria, at which there is no full cellular wall:

- A) Rickettsia;
- B) Mycoplasmas;
- C) Chlamydia;
- D) Spirochetes.

5. Curled bacteria:

- A) Actinomycetes;
- B) Spirilla;
- B) Mycobacteria;
- D) Spirochetes.

6. Actinomycetes:

- A) Gram positive microbes;
- B) Cells have view branched threads;
- C) form exospores;
- D) Prokaryotes.

7. Microorganisms, partially or completely lost cellular wall under action factors external environments:

- A) spheroplasts;
- B) Protoplasts;
- C) L-shape;
- D) Mycoplasmas.

8. Functions LPS:

- A) antigenic;
- B) Enzymatic;
- C) Toxic (endotoxin);
- D) Hereditary.

9. Microbes in which cell wall rigidity causes peptidoglycan:

- A) Gram-negative bacteria;
- B) Viruses;
- B) Gram-positive bacteria;
- D) Mushrooms.

10. Acid resistant microorganisms:

- A) Mycobacteria;
- B) Streptococci;
- C) Vibrios;
- D) Staphylococci.

- 11. Functions pili (villi, fimbria):**
 - A) adhesion of bacteria to the substrate;
 - B) Participation in transfer genes;
 - C) Serve receptor for bacteriophages;
 - D) Are antigens.
- 12. Education endospore at bacteria stimulate:**
 - A) Flaw oxygen;
 - B) Change in ambient temperature;
 - C) deficit nutritional substances;
 - D) hit in organism human or animal.
- 13. signs gram-positive bacteria:**
 - A) There are teichoic acids in the cell wall;
 - B) Can form disputes;
 - C) The main component of the cell wall is peptidoglycan;
 - D) Individual representatives acid resistant.
- 14. What kind peculiarities characteristic for mesosomes at bacteria?**
 - A) are formed as a result of invagination of the cytoplasmic membranes in cytoplasm;
 - B) Performs functions digestive vacuoles;
 - C) Synthesize protein;
 - D) Reveal on Ziel-Nielsen.
- 15. For detection capsules bacteria in clean culture use coloring:**
 - A) simple;
 - B) According to Neisser;
 - C) By Gram;
 - D) By Burri Guinsu.
- 16. What such transformation?**
 - A) Broadcast genetic information at contact bacterial cells different "sexual" orientation;
 - B) Recovery damaged DNA;
 - C) The transfer of genetic material through highly polymerized DNA.
- 17. higher mushrooms:**
 - A) have axial a thread;
 - B) Have septate mycelium;
 - C) Form vegetative endospores;
 - D) Form exospores (conidia).
- 18. Give characteristic protozoa:**
 - A) have a cellular structure;
 - B) Refer to eukaryotes;
 - C) Outside surrounded by pellicle;
 - D) Relate to kingdom animals.
- 19. To microorganisms with a prokaryotic type of cell organization include: a) mold fungi; b) spirochetes; c) chlamydia; D) mycoplasmas; e) actinomycetes.**

Choose the only combination that takes into account all correct answers:

 - A) a, b, in;
 - B) b, in, G, d;
 - B) a in, G, d;
 - D) a, b, G, d;

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

- 1. For detection acid resistant bacteria apply?**
- 2. For detection grains volutin bacteria?**
 - A) Coloring on Buri-Ginsu;
 - B) Coloring on Ziel-Nielsen;
 - C) Coloring on Romanovsky-Giemsa;
 - D) Coloring divorced carbolic magenta;
 - E) coloring by Neisser.

21.

- 1. The function of protein synthesis performs:**
- 2. chromosomal genetic structures at bacteria:**
 - A) mesosomes;
 - B) Ribosomes;
 - C) Plasmids;
 - D) transposons;
 - E) Nucleoid.

«GENERAL MICROBIOLOGY. STRUCTURE OF A BACTERIAL CELL»

IV OPTION

(Choose one or some correct answers)

- 1. Who is one from founders immunological stagedevelopment of microbiology and the creator of the phagocytic theory immunity?**
 - A) Rarely;
 - B) Mechnikov;
 - C) Koch;
 - D) Pasteur.
- 2. What kind microbes relate to eukaryotes?**
 - A) the simplest;
 - B) Mycoplasmas;
 - C) Mushrooms;
 - D) Chlamydia.
- 3. Structural features of prokaryotes:**
 - A) ribosome sedimentation constant 70S;
 - B) Available nucleoid;
 - C) there is no Golgi apparatus;
 - D) Absent nuclear membrane.
- 4. What such streptobacilli?**
 - A) Cocci forming a chain;
 - B) sticks, generators chain;
 - C) Collection forms;
 - D) spore-forming sticks, conducive chain.
- 5. branching forms bacteria:**
 - A) Actinomycetes;
 - B) Spirilla;
 - B) Mycoplasmas;
 - D) Spirochetes.
- 6. Microorganisms in which the absence of a cell wall is always determined genetically:**

- A) protoplasts
- B) Spheroplasts
- C) Chlamydia
- D) Mycoplasmas

7. What kind properties characteristic for chlamydia?

- A) Gram-negative;
- B) Prokaryotes;
- C) Obligate intracellular parasites;
- D) Have twisted shape.

8. Lipopolysaccharide bacterial cells situated in:

- A) cytoplasmic membrane;
- B) outdoor membrane gram-positive bacteria;
- C) Mesosomes;
- D) outdoor membrane Gram-negative bacteria.

9. AT composition peptidoglycan includes:

- A) Teichoic acids
- B) N-acetylglucosamine and M-acetylmuramic acid
- C) Lipopolysaccharide (LPS)
- D) glycan molecules.

10. What kind structures required for L-shape bacteria?

- A) a capsule
- B) CPM;
- B) cytoplasm;
- D) Nucleoid;
- E) Cellular wall.

11. Non-spore-forming bacteria, most sustainable to action acids, alkalis and alcohol:

- A) Mycobacteria;
- B) Clostridia;
- B) Escherichia;
- D) Bacillus.

12. Intracellular inclusion bacteria:

- A) Glycogen grains;
- B) Mitochondria;
- C) grains of volutin;
- D) Ribosomes.

13. Complex differential diagnostic methods coloring:

- A) Tsil-Nelsen stain;
- B) Coloring blue Leffler;
- C) coloring by Gram;
- D) Coloring divorced carbolic fuchsin.

14. On microscopy of the test material, rickettsia usually discover:

- A) AT cytoplasmic membrane;
- B) AT mesosomes;
- C) extracellular;
- D) AT cytoplasm cells.

15. For detection grains volutin at bacteria coloring is used:

- A) By Neisser;
- B) According to Romanovsky-Giemsa;
- C) According to Burri-Guins;
- D) By Ozheshki.

16. What such mutagens?

- A) genes, providing mutation
- B) Factors defiant mutation
- C) Factors regenerating DNA;
- D) Fatory, transmitting genetic information.

17. Mycelium mushrooms - this is:

- A) Cell without cytoplasmic membranes;
- B) Aggregate hyphae;
- C) The totality of chlamydospores;
- D) multi-core structure.

18. Protozoa:

- A) eukaryotes;
- B) Contain a well-formed nucleus with a nuclear membrane;
- C) Arranged more difficult, how cells bacteria;
- D) Prokaryotes.

19. To microorganisms with a prokaryotic type of cell organization include: a) chlamydia; b) viruses; c) mold fungi; D) spirochetes; e) actinomycetes; e) mycoplasmas.

Choose the only combination that takes into account all correct answers:

- A) a b, in;
- B) a, g, d, e;
- C) in, G, d;
- D) a, in, G, d;
- E) b, G, d.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

20.

1. Transduction:

2. Conjugation:

- A) Correction damaged plots DNA;
- B) Transfer of genetic information with the help of a bacteriophage;
- C) hereditary hop change sign;
- D) Broadcast genetic information at crossing bacterium through genital villi.
- E) Transfer of genetic material using highly polymerized DNA.

21.

1. Actinomycetes:

2. Chlamydia:

- A) Gram-positive microbes;
- B) Gram negative microbes;
- C) Cells look like branched filaments;
- D) form exospores;
- D) Obligate intracellular parasites.
- E) Eukaryotes.

«INFECTION AND IMMUNITY. STRUCTURE AND FUNCTIONS OF ANTIGENS AND ANTIBODIES»

I OPTION

(Choose one or some correct answers)

1. Microbe pathogenicity - this is sign:

- A. Genotypic;
- B. Potential;
- C. Inherent mind microbe;
- D Influencing susceptibility macroorganism.

2. Dlm is unit measurements:

- A. Virulence of microbes;
- B. Antigenicity of microbes;
- C. Toxigenicity microbes;
- D. Immunogenicity microbes.

3. characteristic properties endotoxin:

- A. Protein nature;
- B. Causes an increase in body temperature;
- C translated in toxoid;
- G. Is factor pathogenicity.

4. characteristic signs infectious diseases:

- A. The presence of a microbe-causative agent;B. contagiousness;
- B. Formation of the immune response;
- G. cyclicity currents.

5. For septicopyemia characteristic:

- A. Hematogenous spread of bacteria in the macroorganism;
- B. reproduction bacteria in blood;
- G. Formation of secondary purulent foci in the internal organs;
- D. Circulation microbial toxins in blood.

6. name form infectious process, at which pathogen prolonged time is in body, not showing pathogenic properties and not standing out in environment:

- A. Bacteriocarrier;
- B. Latent infection;
- C. Slow infection;
- G. Acute infection.

7. Naturally acquired immunity:

- A. After the introduction of immune sera;
- B. post-infectious;
- C. Post-vaccination;
- G. Transplacental.

8. Acquired artificial active immunity:

- A. After the introduction of antitoxic serum;
- B. Post-vaccination;
- C. Transplacental;
- D Post-infectious.

9. Name the process that protects the body from repeated antigenic interventions:

- A. Immune tolerance;
- B. immune memory;
- C. Hypersensitivity;
- D. Immune paralysis.

10. Alternative way activation complement starts:

- A. Histamine;
- B. Components of the bacterial cell wall;
- C. Complex "antigen-antibody";
- D. Lipopolysaccharide.

11. Immunoglobulin class G:

- A. Binds complement;
- B. Found in mucous secretions;
- C. passes through placenta;
- D. Provides local immunity.

12. Antibodies:

- A. Synthesized plasma cells;
- B. Able bind complement;
- C. Able neutralize toxins;
- D. Agglutinate corpuscular antigens.

13. Check effector cells immune systems:

- A. T-killers;
- B. T-helpers;
- C. Dendritic cells;
- D. B-lymphocytes.

14. Phenomenon immunological memory based on:

- A. oppression T-helpers;
- B. The absence of certain clones of immune cells;
- C. Absence antigens histocompatibility;
- D. Cell formation memory.

15. name signs hypersensitivity delayed type:

- A. Lymphocyte-macrophage reaction;
- B. Ig synthesis E;
- C. Participation of T-lymphocytes;
- D. Participation B-lymphocytes.

16. Immunomodulators:

- A. Influence the pathological process through the genome;
- B. Possess immunotropic action;
- C. Influence the pathological process through the immune system;
- D. AT basis mechanism actions lie immunological reactions.

17. Antitoxic immunity:

- A. Absorption toxin by macrophages;
- B. Development of antitoxic antigens;
- C. Activation T-killers;
- D. Production antitoxic antibodies.

18. Purpose RPGA:

- A. Serodiagnosis of infectious diseases;
- B. Identification microbe;
- C. Determination of specific antibodies;
- D. Titration complement.

MAKE LOGICAL PAIRS: QUESTION AND ANSWER19 .

1. relapse

2. reinfection

3. Superinfection

- A. Disease, arising after transferred infections per check repeated

- infections topics same pathogen;
- B. Repeat infection macroorganism topics same pathogen before recovery;
- C. Both;
- D. Neither then, neither other.

20.

1. Passive, naturally acquired immunity

2. Active, naturally acquired immunity

- A. post-infectious;
- B. Post-vaccination;
- C. Transplacental;
- D. Transplant.

21.

1. Antigen in reactions agglutination

2. Antigen in reactions precipitation

- A) Molecular;
- B. Corpuscular;
- C. Both;
- G. Neither then, neither other.

«INFECTION AND IMMUNITY. STRUCTURE AND FUNCTIONS OF ANTIGENS AND ANTIBODIES»

I OPTION

(Choose one or some correct answers)

1. Virulence microbes:

- A. Controlled by the genes of the chromosome and plasmids;
- B. Determine on sensitive animals;
- B. Changes under the influence of external factors;
- G. Is specific sign.

2. Adhesins microbes:

- A. Hyaluronidase;
- B. Endotoxins;
- C. Exotoxins;
- G. We drank.

3. Properties bacterial endotoxins:

- A) Lipopolysaccharide nature;
- B. They are excreted by bacteria in the process of life;
- B. Not has specificity actions in body;
- G. Under action formalin are turning in toxoid.

4. Periods in development infectious process

- A) Premonitory;
- B. Reconvalescence;
- C. Incubation;
- G. Superinfection.

5. Forms infections:

- A) reinfection;
- B. Reconvalescence;
- C. relapse;

G. Incubation.

6. Author phagocytic theories immunity:

A. Burnet F.;

B. erne N.;

C. Erlich P.;

G. Mechnikov I.I.

7. Artificially acquired immunity:

A. After the introduction of immune sera;

B. post-infectious;

B. Post-vaccination;

G. Transplacental.

8. Complete antigens:

A. Specific;

B. Interact with specific antibodies;

C. Have high molecular mass;

G. Possess immunogenicity.

9. AT structure bacterial cells may include antigens:

A. H-antigen;

B. K-antigen;

C. O antigen;

G. HLA antigens.

10. Biological liquids, in which contained lysozyme:

A) Tears;

B. Tissue fluid;

C. Saliva;

G. Serum.

11. Interferons:

A. Produced by fibroblasts and T-lymphocytes;

B. Produced leukocytes;

B. They have immunomodulatory properties;

G. Possess specific specificity.

12. Immunoglobulin class M:

A) binds complement;

B. Passes through the
placenta;

C. Pentamer;

G. It has 2 center antigen binding.

13. Secretory immunoglobulin class A:

A. Provides local immunity;

B. Is pentamer;

B. Contains a secretory component;

G. passes through placenta.

14. Local provide immunity immunoglobulins:

A. Class G;

B. Class E;

B. Class D;

G. Class A

15. phagocytes may to be cells:

A) Monocyte;

B. Neutrophil;

B. Alveolar macrophage;

G. Erythrocyte.

16. Specify forms immunity, in which accepts participation

complement:

- A. Mucosal immunity;
- B. Antitoxic;
- C. Antibacterial humoral;
- G. Humoral viral.

17. Specify the forms of immunity in which T-killers:

- A. Transplant;
- B. Antitumor;
- C. Antiviral;
- G. Antibacterial.

18. List Components RPGA:

- A. red blood cells;
- B. Erythrocyte diagnosticum;
- C. Hemolytic serum;
- D. Researched serum.

**MAKE LOGICAL PAIRS: QUESTION AND ANSWER
nineteen.**

1. Exotoxins

2. Endotoxins

3. Anatoxins

- A. Do not have toxic properties;
- B. stand out microbe in environment;
- D. Released when bacteria are destroyed
- G. Neither then, neither other.

20.

1. Complete antigen

2. Defective antigen

- A. Polysaccharide;
- B. Protein;
- C. Both;
- G. Neither then, neither other.

21.

1. Definition molecular antigens

2. Definition corpuscular antigens

- A. Precipitation reaction;
- B. Agglutination reaction;
- C. Both;
- G. Neither then, neither other.

**«INFECTION AND IMMUNITY. STRUCTURE AND FUNCTIONS OF ANTIGENS AND
ANTIBODIES»**

I OPTION

(Choose one or some correct answers)

1. Pathogenicity of microbes - this is sign:

- A. Species;
- B. Arose in the process of evolution of parasitism;
- C. Genotypic;
- D. Changes quickly under influence factors environment.

2. adhesive ability bacteria due to:

- A. The presence pili;

- B. The presence peptidoglycan;
- C. The presence of lipoteichoic acids;
- G. education protein toxins.

3. characteristic properties endotoxins:

- A. Strong antigens;
- B. They are found in the cell wall of gram-negative bacteria;
- C. Thermolabile;
- G. Not sensitive to formalin.

4. Premonitory period - this is period:

- A. From the moment of infection to the onset of clinical manifestations of the disease;
- B. intensive breeding pathogen in place input gate;
- C. Liberations macroorganism from microbes;
- G. Appearances non-specific symptoms infectious illness.

5. Repeated manifestations diseases, caused those same pathogens:

- A. Relapse;
- B. Secondary infection;
- C. reinfection;
- G. Mixed infection.

6. Author humoral theories immunity:

- A. Burnet F.
- B. Erne N.
- C. Mechnikov I.I.
- D Erlich P.

7. Active immunity:

- A. After the introduction of immune sera;
- B. Post-vaccination;
- B. Transplacental;
- G. Post-infectious.

8. Chemical substances being full-fledged antigens:

- A) Protein;
- B. mineral salt;
- C. Polysaccharide;
- G. Lipid.

9. To factors non-specific resistance relate:

- A. Phagocytosis;
- B. Lysozyme;
- C. Complement;
- D. Normal microflora.

10. Interferons:

- A. Produced by fibroblasts and T-lymphocytes;
- B. Produced leukocytes;
- C. They have immunomodulatory properties;
- G. Possess specific specificity.

11. Immunoglobulin class M:

- A. binds complement;
- B. Passes through the placenta;
- C. Pentamer;
- D. It has 2 center antigen binding.

12. Immunoglobulin class E:

- A. Passes through the placenta;
- B. Pentamer;

- C. Provides local immunity;
- D. Possesses cytophilicity to mast cells and basophils;

13. Monoclonal antibodies:

- A. Possess heterogeneity;
- B. Are synthesized hybridoma;
- B. Synthesized in the human body;
- G. High specific.

14. In the formation of nonspecific resistance are involved cells:

- A. T-helpers;
- B. macrophages;
- C. B-lymphocytes;
- D. natural killers.

15. Functions T-helpers:

- A. Production of antibodies;
- B. Phagocytosis;
- C. The manifestation of cytotoxicity;
- D. Regulation immune response.

16. Neutralization virus outside cells (virion) carried out:

- A. Class A immunoglobulins;
- B. Interferons;
- C. Class G immunoglobulins;
- G. T cells.

17. For creation artificial active immunity use:

- A. Vaccines;
- B. Immune sera;
- C. Anatoxins;
- G. Tolerogens.

18. List Components reactions precipitation:

- A) red blood cells;
- B. Molecular antigen;
- C. Hemolytic serum;
- G. Specific immune serum.

MAKE LOGICAL PAIRS: QUESTION AND ANSWER nineteen.

1. aerogenic mechanism transmission pathogen

2. fecal-oral mechanism transmission pathogen

3. Transmissible mechanism transmission pathogen

- A) Transfer pathogen through allocation his With faeces and penetration in organism through gastrointestinal tract;
- B. Transfer of the pathogen through blood-sucking arthropods;
- C. Both;
- G. Neither then, neither other.

20.

1. cells, not having antigens histocompatibility

2. cells, having antigens histocompatibility

- A. Lymphocytes;
- B. Erythrocytes;
- C. Both;
- G. Neither then, neither other.

21.

1. Titer agglutinating serum

2. Titer hemolytic serum

- A. The highest dilution of serum that causes complete lysis of erythrocytes in presence complement;
- B. The minimum serum dilution at which hemolysis occurs;
- C. Both;
- G. Neither then, neither other.

«INFECTION AND IMMUNITY. STRUCTURE AND FUNCTIONS OF ANTIGENS AND ANTIBODIES»

IV OPTION

(Choose one or some correct answers)

1. Factors conditioning pathogenicity microbes:

- A. Production of aggression enzymes;
- B. Toxin formation;
- B. Capsule formation;
- G. Availability adhesins.

2. Factors pathogenicity bacteria With invasive function:

- A. Membranotoxins;
- B. Hyaluronidase;
- B. Capsule;
- G. Neuraminidase.

3. Describe protein toxins bacteria:

- A) Are synthesized gram-positive bacteria;
- B. Are released into the environment in the process of life;
- C. Can partially be secreted;
- G. Not possess specificity actions.

4. name forms infections on sign localization pathogen:

- A. Manifesto;
- B. Sepsis;
- C. relapse;
- G. Septicopyemia.

5. Forms of infections characterized by long stay microbes in macroorganism:

- A. Bacteriocarrier;
- B. persistence;
- C. relapse;
- G. Secondary infection.

6. Peripheral bodies immune systems:

- A. Bone marrow;
- B. thymus;
- C. Plasma cells;
- G. Lymphatic nodes.

7. Passive immunity:

- A. After the introduction of immune sera;
- B. Post-vaccination;
- B. Transplacental;
- G. Post-infectious.

8. Haptens:

- A) Are determined in reactions agglutination;

- B. Interact With antibodies;
- B. Induce an immune response in the macroorganism;
- G. Have low molecular mass.

9. Activation complement may begin With component:

- A) C1;
- B. C2;
- B. C3;
- G. C4.

10. Immunoglobulin class M:

- A) binds complement;
- B. Passes through the placenta;
- C. Pentamer;
- G. It has 2 center antigen binding.

11. Immunoglobulin class E has tropism to:

- A) Basophils;
- B. macrophages;
- B. mast cells;
- G. fibroblasts.

12. Monoclonal antibodies:

- A. High specific;
- B. Possess structural heterogeneity;
- B. Used as diagnostic preparations;
- G. Are produced macrophages.

13. Phenomena of the immune response, in which B-lymphocytes:

- A. Production of antibodies;
- B. Phagocytosis;
- B. Immunological memory;
- G. killernaya function

14. Specify immunocompetent cells, possessing cytotoxicity:

- A. Natural killers;
- B. T-helpers;
- B. T-killers;
- G. Basophils.

15. For antibacterial immunity characteristically participation:

- A. Complement;
- B. Phagocytes;
- B. Antibodies;
- D. B-lymphocytes.

16. signs hypersensitivity I type (anaphylaxis):

- A. Immediate reaction development;
- B. Possibility of desensitization;
- C. Participation of B-lymphocytes;
- G. Ig involvement E.

17. For creation artificial passive immunity use:

- A. Vaccines;
- B. Immune sera;
- C. Immunoglobulins;
- D. Adjuvants.

18. Purpose reactions precipitation:

- A. Determination of unknown antibodies against a known antigen;

- B. Definition quantities erythrocytes;
- B. Determination of an unknown antigen by known antibodies;
- G. Definition titra complement.

**MAKE LOGICAL PAIRS: QUESTION AND ANSWER
nineteen.**

- 1. Anthroponosis**
- 2. zooanthroponosis**
- 3. Sapronose**

- A. The source of infection is a person;
- B. The source of infection is an animal;
- C. Both;
- G. Neither then, neither other.

20.

- 1. Flagellate antigen bacteria**
- 2. Somatic antigen bacterial cells**

- A. H-antigen;
- B. O-antigen;
- C. Both;
- G. Neither then, neither other.

21.

- 1. Bacterial diagnosticum**
- 2. Diagnostic serum**

- A. Contains specific antibodies;
- B. Antigen in corpuscular form;
- C. Both;
- G. Neither then, neither other

**«PATHOGENS OF DISEASES CAUSED BY PATHOGENIC COCCAS.PATHOGENS
BACTERIAL INTESTINAL INFECTIONS»**

I OPTION

(Choose one or some correct answers)

1. Purulent-inflammatory diseases, called conditionally pathogenic cocci are characterized by:

- A) Various localization;
- B. The variety of clinical forms;
- C. Decrease resistance macroorganism;
- G. Weak immune answer.

2.Toxoid can be used to prevent what kind of infection?

- A) Staphylococcal;**
- B. Streptococcal;
- C. gonococcal;
- G. Meningococcal.

3. Nutrients environment, which can use for allocation conditionally pathogenic staphylococci:

- A) 10 % yolk - salt agar;
- B. Blood agar;
- C. Whey agar;
- G. MPA.

4. At what diseases applied method "provocations"

- A. Rheumatism;
- B. meningitis;
- C. gonorrhea;

G. Syndrome toxic shock.

5. Which from microorganism coccoid forms produces toxin "syndrome toxic shock":

A. Pneumococcus;

B. Staphylococcus; B.

Streptococcus;

G. Meningococcus.

6. Material for bacteriological method research with meningococcal infections:

A) Liquor;

B. smear from nasopharynx;

C. Blood;

G. Serum

7. Properties bacteria genus Salmonella:

A) produce H₂S ; _

B. Lactose-negative;

C. mobile;

G. Gram-positive.

8. Material for bacteriological research at cholera:

A) Blood;

B. Vomit;

C. Urine;

D. stool;

9. For serological method diagnostics abdominal typhus apply reactions:

A) RNGA;

B. ELISA;

B. PCR;

G. RA on glass.

10. diarrheagenic intestinal sticks:

A. Produce enterotoxins;

B. Lactose-positive;

B. Have pathogenicity plasmids;

G. Have endotoxin.

11. Nutrients environments for allocation and identification pathogen shigellosis:

A. Ploskireva;

B. Kligler;

C. Endo;

G. alkaline peptonic water.

12. Properties bacteria genus Shigella:

A) form disputes;

B. Lactose-negative;

C. Have N- antigen;

G. Not produce H₂S . _

13. Factors pathogenicity pathogens cholera:

A. Invasive outer membrane proteins;

B. Enterotoxin;

C. Toxin Shiga;

G. Neuraminidase.

14. Serological method diagnostics abdominal typhus allows:

A. Assess the dynamics of the disease;

B. Reveal bacteriocarrier;

C. Spend retrospective diagnostics;

G. Define biochemical properties pathogen.

15. Material for bacteriological research on 1st week diseases

abdominal typhus:

- A. Urine;
- B. Excreta;
- C. Serum;
- D. Blood.

16. Useful functions intestinal sticks for macroorganism:

- A. Antagonist of pathogenic putrefactive microflora;
- B. Not splits fiber;
- B. Participate in the synthesis of vitamins;
- G. Partially splits fiber.

17. Methods of microbiological diagnosis of typhoid fever at the 3rd weekdiseases:

- A. Bacterioscopic;
- B. Bacteriological;
- C. Biological;
- G. Serological.

18. Development diarrheal syndrome at salmonellosis is result:

- A) Actions enterotoxin;
- B. Reproduction of Salmonella in epithelial cells of the surface epithelium;
- C. Activations endotoxin arachidonic cascade acids;
- G. Actions Shiga-like toxin.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

19.

- 1. Unfinished phagocytosis:**
- 2. Forms chains cells in bouillon culture:**
- 3. Maybe produce enterotoxin:**
- 4. calls blennorey:**
 - A. S. aureus;
 - B. S.pyogenes;
 - C. N. gonorrhoeae.

20.

- 1. Cholera:**
- 2. Shigellosis:**
- 3. Salmonellosis:**
- 4. Intestinal escherichiosis:**
 - A.ETKP;
 - B. S enteritidis;
 - C. S.typhi;
 - G. V.cholerae;
 - D. S. sonnei.

21.

- 1. Agglutinated by polyvalent escherichial OK-serum(antibodies to 0157):**
- 2. Cause purulent-inflammatory diseases of variouslocalization:**
- 3. produce enterotoxins:**
- 4. Possesses psychrophilia:**
 - A. Conditionally pathogenic Escherichia coli;
 - B. Diarrheic Escherichia coli;
 - B. Both;
 - G. Neither then, nothing else.

«PATHOGENS OF DISEASES CAUSED BY PATHOGENIC COCCAS.PATHOGENS BACTERIAL INTESTINAL INFECTIONS»

I OPTION

(Choose one or some correct answers)

1. Gonococci and meningococci in clean culture and researched material usually located:

- A. Single;
- B. Chains;
- C. in pairs;
- G. Clusters.

2. Material for bacteriological research at scarlet fever:

- A. Blood;
- B. Urine;
- C. Serum;
- G. Smear from pharynx.

3. antigens staphylococci are:

- A) Protein A;
- B. Teichoic acids;
- C. toxins;
- G. Lipopolysaccharide.

4. For treatment: heavy acute staphylococcal infections (sepsis and etc.) can use:

- A. Immunoglobulin;
- B. killed vaccine;
- C. Hyperimmune plasma;
- G. Live vaccine.

5. For what pathogen characterized by unfinished phagocytosis?

- A) Golden staphylococcus;
- B. Streptococcus;
- C. epidermal staphylococcus;
- G. Gonococcus.

6. At what forms may be leak meningococcal infection?

- A) Meningitis;
- B. Nasopharyngitis;
- C. "Healthy" carriage;
- G. Furunculosis.

7. What kind methods are used for diagnostics abdominal typhus?

- A. Bacterioscopic;
- B. Bacteriological;
- C. Biological;
- G. Serological.

8. Properties bacteria kind Escherichia:

- A) Gram-positive;
- B. Lactose-positive;
- C. form disputes;
- G. Not produce H₂S.

9. What properties possess bacteria family Enterobacteriaceae:

- A) Gram negative sticks;
- B. Not form dispute;
- B. Facultative anaerobes;
- G. Have grain volutin.

10. name factors pathogenicity shigella:

- A. Invasive outer membrane proteins;
- B. W, V antigens;
- B. Shiga-like toxin;
- G. Cholera toxin.

11. What factors pathogenicity possesses pathogen cholera:

- A. Invasive outer membrane proteins;

- B. Enterotoxin;
- C. Toxin Shiga;
- G. Neuraminidase.

12. Serological method diagnostics abdominal typhus allows:

- A) Estimate dynamics infectious process;
- B. Reveal bacteriocarrier;
- B. Conduct a retrospective diagnosis;
- G. Seropite pathogen.

13. What kind environments use at allocation pathogen cholera?

- A. Alkaline peptone water;
- B. Kligler;
- C. Alkaline agar;
- G. biliary bouillon.

14. By what properties differ diarrheagenic intestinal sticks?

- A. Presence of virulence plasmids;
- B. lactose-negative;
- B. Antigenic structure;
- G. Products H₂S . _

15. Name drugs for specific prevention abdominal typhus:

- A) Chemical vaccine;
- B. inactivated corpuscular vaccine;
- C. Bacteriophage;
- G. Anatoxin.

16. What kind drugs are used for treatment and prevention dysentery?

- A) Intesti bacteriophage;
- B. Dysenteric bacteriophage;
- C. Vi bacteriophage;
- G. Pyocyneus bacteriophage.

17. What kind methods diagnostics cholera relate to accelerated?

- A. Immobilization in a dark field;
- B. Dark field agglutination;
- C. Method Ermolyeva;
- G. Immunofluorescent method.

18. . Name e gray vars ho lern og about inibrion?

- A) Og aw a;
- B. Inaba;
- C. Gikoshim a;
- G. Holeresui With.

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

19.

1. Lecitovitellase activity on yolk-salt agar:
2. Availability plasma coagulase:
3. Absence plasma coagulase:

4. Pigmentation:

- A. Staphylococcus aureus;B.
- Streptococci;
- B. Both;
- G. Neither then, neither other.

20.

1. Cholera:
2. Paratyphoid AND:
3. Intestinal escherichiosis:
4. Shigellosis:
 - A. S. dysenteriae;
 - B. V. cholerae;
 - B. S. typhimurium;

- G. EPPK;
- D. S. paratyphi.

21.

1. Applies to serogroup O1:
2. sustainable to polymyxin:
3. sensitive to bacteriophage FROM:
4. Produces enterotoxin:
 - A. biovar cholerae;
 - B. Biovar eltor;
 - C. Both;
 - G. Neither then, neither other.

«PATHOGENS OF DISEASES CAUSED BY PATHOGENIC COCCAS.PATHOGENS BACTERIAL
INTESTINAL INFECTIONS»

I OPTION

(Choose one or some correct answers)

1. Methods of microbiological diagnosis of pneumococcal infection :A)

Bacteriological;

- B. Serological;
- B. Biological;
- G. Allergic.

2. What bacteria are located in pure culture and test material?in pairs?

- A. Pneumococci;
- B. Staphylococci;
- C. meningococci;
- G. Everybody the above right.

3. What material for microbiological research should be taken from the patient at suspicion for gonorrhea?

- A. Detachable urethra;
- B. Vaginal swab;
- C. Smear from pharynx;
- G. Rectal smear.

4. After what diseases streptococcal etiology formed lastingimmunity?

- A. Tonsillitis;B.
- Rheumatism;
- B. Scarlet fever;
- G. Sepsis.

5. name toxin pathogen scarlet fever:

- A. Fibrinolysin;
- B. Erythroginin;
- C. Erythrolysin;
- G. Plasmocoagulase.

6. Basic way transmission blenorei newborns:

- A) Contact;
- B. Contact household;
- C. Sexual;
- G. Water.

7. What kind properties characteristic for representatives families Enterobacteriaceae:

- A. They need alkaline nutrient media;B. Gram negative sticks;
- B. form disputes;
- G. fermented carbohydrates.

8. What media are used to isolate and identify the pathogencolienteritis?

- A) Endo;
- B. Kligler;
- C. Levin;
- G. biliary bouillon

9. reactions, used for serological method diagnostics abdominal typhus:

- A) RINGA;
- B. ELISA;
- C. deployed RA;
- G. RA on glass.

10. By what properties differ biovars vibrio cholerae and eltor?

- A. According to the agglutination reaction with 01 - serum;
- B. By sensitivity to polymyxin;
- C. By relation to serum Inaba;
- G. By sensitivity to specific bacteriophages.

11. Development diarrheal syndrome at salmonellosis related With:

- A) action enterotoxin;
- B. Reproduction of salmonella in epithelial cells of the surface epithelium;
- C. Activation endotoxin arachidonic cascade acids;
- G. blocking neurovascular receptors.

12. Preparations for specific prevention abdominal typhus:

- A) Chemical vaccine;
- B. inactivated corpuscular vaccine;
- C. Bacteriophage;
- G. Anatoxin.

13. Material for bacteriological research at cholera:

- A) Blood;
- B. Vomit;
- C. Urine;
- D. stool;

COMPOSE BRAIN TEASER COUPLES: QUESTION ANSWER

14.

- 1. Absence tropism
- 2. Low enzymatic activity
- 3. High enzymatic activity
- 4. Height on saline environments
- 5. Height only on rich protein environments
 - A. Pathogenic Neisseria;B. Staphylococci;
 - B. Both;
 - G. Neither then, neither other.

20. Transcytosis epithelium thin guts With breeding in regionallymphoid fabrics intestines:

- 1. Invasion and intracellular reproduction in the epithelium of the colon intestines:
- 2. attachment and colonization surfaces epithelium thin intestines:A) Shigella;
 - B. Salmonella;
 - B. Vibrio cholerae;G. EPKP.

21.

- 1. split mannitol:
- 2. More often transmitted water by:
- 3. More often transmitted contact household by:
- 4. Reproduces in intestinal tissue:A) S. flexneri;
 - B. S. dysenteriae;
 - B. Both;
 - G. Neither then, nothing else.

«PATHOGENS OF DISEASES CAUSED BY PATHOGENIC COCCAS.PATHOGENS BACTERIAL
INTESTINAL INFECTIONS"

II OPTION

(Choose one or several correct answers)

1. As if pathogenic cocci that can cause diseases of various localization:

- A. Staphylococci;
- B. Pneumococcus;
- B. Streptococci;
- G. Meningococcus.

2. Biological properties pathogen gonorrhea:

- A. Sensitive to environmental factors;
- B. pathogenic only for person;
- V. Grows on nutritional environments With adding human squirrel;
- G. Mobile.

3. Main methods diagnostics pneumococcal infections:

- A) Allergic;
- B. Bacteriological;
- C. Serological;
- G. Biological.

4. Multidrug resistance in staphylococci is due to presence:

- A) Capsules;
- B. Ent-plasmids;
- B. Hyaluronidase;
- G. R-plasmids.

5. What kind cocci sensitive to optochin and bile:

- A. Streptococcus;
- B. Staphylococcus;
- C. Gonococcus;
- G. Meningococcus.

6. For -hemolytic streptococci characteristic:

- A. They have adhesins - a complex of lipoteichoic acid;
- B. motionless, dispute and capsules not forms;
- B. They produce enzymes: streptokinase and hyaluronidase;
- G. Allocate toxins .

7. What kind antigens virulence eat at E.coli?

- A) E;
- B. W;
- C. H;
- G. K;
- D. Oh

8. What properties possess bacteria kind Shigella?

- A) form disputes;
- B. Lactose-negative;
- C. Possess H-antigen;
- G. Not produce H₂S . _

9. What kind vaccines used for specific prevention abdominal typhus?

- A. O-vaccines;
- B. AKSD vaccines;
- C. Typho-paratyphoid-tetanus vaccines;
- G. BCG.

10. Pathogen abdominal typhus strikes:

- A. The mucous membrane of the stomach;
- B. Epithelium of the small intestine;C.
- Heart;

G. Kidneys.

11. causative agents salmonellosis are:

- A. S. enterica;
- B. S. typhi;
- C. S. typhimurium;
- G.S. enteritidis.

12. Nutrients environments for allocation and identification pathogen shigellosis:

- A. Ploskireva;
- B. Kligler;
- C. Endo;
- G. alkaline peptonic water.

13. Methods microbiological diagnostics abdominal typhus on 3 week diseases:

- A. Bacterioscopic;
- B. Bacteriological;
- C. Biological;
- G. Serological.

14. Serological method diagnostics abdominal typhus allows:

- A. Assess the dynamics of the disease;B.
- Reveal bacteriocarrier;
- C. Spend retrospective diagnostics;
- G. Serotype pathogen.

15. Material for bacteriological research at shigellosis:

- A. Blood;
- B. Urine;
- C. Excreta;
- G. Serum.

16. What kind environments use for accumulation vibrio cholerae?

- A. Sugar broth;
- B. biliary bouillon;
- B. Serum bouillon;
- G. alkaline peptonic water.

17. What kind antigens have salmonella abdominal typhus?

- A) O;
- B. H;
- B. Vi;
- G. TO;
- D.W.

18. For serological method diagnostics abdominal typhus apply reactions:

- A) RNGA;
- B. ELISA;
- B. PCR;
- G. RA on glass.

LEAVE BRAIN TEASER COUPLES: QUESTION ANSWER

19.

- 1. erysipelatus inflammation:
- 2. Blennorea:
- 3. Syndrome toxic shock:
- 4. Rheumatism:

- A. Staphylococcus
- ;B. Gonococcus;
- B. Group A beta-hemolytic streptococci;G.
- Pneumococcus.

20.

- 1. attachment and colonization surfaces epithelium thin intestines:
- 2. Transcytosis epithelium thin guts With breeding in regional lymphoidfabrics intestines:

3. Invasion and intracellular reproduction in epithelium thick intestines:

- A) Shigella;
- B. Salmonella;
- V. cholera vibrio;

21.

1. Basic way transmission - contact household:
2. Basic way transmission – water:
3. Produces a Shiga-like toxin:
4. Works out Shiga -toxin:
 - A.S. sonnei;
 - B. S. dysenteriae;C.
 - Both;
 - G. Neither then, neither other.

"THE CAUSES OF DIPHTHERIA, WHOOPING COUGH, PARACROUP, TUBERCULOSIS, ZOONOUS INFECTIONS»

I OPTION

(Choose one or some correct answers)

1. What form may have pathogen diphtheria?

- A) coccoid;
- B. Polymorphic sticks;
- B. Curved (2-3 curls);
- G. Branching.

2. Microscopy pathogen diphtheria carry out:

- A. When stained according to Tsil - Nelsen;
- B. AT dark field vision;
- B. When stained according to Neisser;
- G. In a negative way.

3. For coloring mycobacteria use method:

- A) Ozheshko;
- B. Tsilya - Nelsen;
- C. Leffler;
- G. Romanovsky-Giemsa;D. Neisser.

4. Sequence stages bacteriological method research at diphtheria:

- A) Definition toxicity;
- B. Sowing the test material on special media;
- C. The study of biochemical properties;
- G. Reseeding colonies for receiving clean culture.

5. Toxicity diphtheria sticks define With help reactions:

- A. Agglutinations on glass;
- B. hemagglutination;
- B. Ring precipitation;
- D. Precipitation in gel.

6. name main methods microbiological diagnostics diphtheria:

- A. Microscopic;
- B. Biological;
- B. Bacteriological;
- G. Allergic.

7. Methods microbiological diagnostics whooping cough

- A. Bacterioscopic;
- B. Bacteriological;

- C. Allergic;
G. Serological.
- 8.** Which method use for accelerated bacteriological diagnostic tuberculosis?
A) Homogenization;
B. Microcultivation;
C. precipitation;
D. Method Price.
- 9.** Vaccine for specific prevention tuberculosis:
A. Killed; B. Live;
B. Anatoxin;
G. BCG.
- 10.** Distinguish pathogen tuberculosis from pathogen leprosy at carrying out microbiological diagnostics can on:
A) acid resistance;
B. Growth on artificial nutritional environments;
C. results PCR;
G. results bioassays.
- 11.** For prevention leprosy apply:
A. Dry purified tuberculin;
B. Integral lepromin;
B. DPT; G. BCG.
- 12.** For serodiagnosis brucellosis apply:
A) reaction Vidal;
B. reaction Wright;
B. Weil-Felix reaction;
G. ELISA.
- 13.** For serodiagnosis tularemia apply:
A) RNGA;
B. RSK;
C. REEF;
G. deployed RA.
- 14.** Nutrients environments for cultivation anthrax bacilli:
A) JSA;
B. Alkaline agar;
C. biliary bouillon;
G. MPA.
- 15.** Express diagnosis of plague:
A. Gas-liquid chromatography;
B. REEF;
B. Phage typing;
G. Phage diagnostics.
- 16.** Vaccines for prevention zoonotic bacterial infections:
A) killed;
B. Anatoxin;
B. Chemical;
G. Live.
- 17.** The form plague, source infections at which is only human:
A) bubonic;
B. Intestinal;
C. Skin-bubonic;
G. Pulmonary.
- 18.** Infection people brucellosis happens :
A. In contact with sick animals;
B. Through milk and dairy products;
C. Through postpartum discharge;

G. At contact With sick people.

19. Make up brain teaser couples: question answer

- | | |
|--------------------------|--|
| 1. split urea | A. Pathogen diphtheria |
| 2. Not possess cystinase | b. Conditionally pathogenic corynebacteria |
| 3. Not have urease | B. Both |
| 4. Work out cystinase | G. Neither not other |

20. Compose brain teaser couples: question answer

- | | |
|--------------------|--|
| 1. M. leprae | A. They are located intracellularly, forming balls |
| 2. M. bovis | B. Gram negative cocci |
| 3. M. tuberculosis | B. Long thin sticks |
| | G. Short thick sticks |

21. Make up brain teaser couples: question answer

- | | |
|-------------------------|--------------------------------|
| 1. Gram positive sticks | A. Pathogen Siberian ulcers |
| 2. Gram negative sticks | B. Pathogen brucellosis |
| 3. Maybe form capsule | B. Both |
| 4. moving | G. Neither then, neither other |

"THE CAUSES OF DIPHTHERIA, WHOOPING COUGH, PARACCOUSH, TUBERCULOSIS, ZOONOUS INFECTIONS»

I

OPTION

(Choose one or some correct answers

1. What morphological structures has pathogen diphtheria?

- A. Spores;
- B. Pilyami;
- C. flagella;
- G. grains volutin.

2. Ways transmission pathogen tuberculosis:

- A. Airborne;
- B. Sexual;
- B. Air and dust;
- G. Transmissible.

3.name main sources tuberculosis:

- A. Patients with an open form of tuberculosis;
- B. Patients with a closed form of tuberculosis;
- B. Patients farm animals;
- G. Marine pigs.

4. Which material take on study at pulmonary forms tuberculosis?

- A) Sputum;
- B. Pleural liquid;
- B. Flushing water of the bronchi;
- G. ascitic liquid.

5. diseases, called mycobacteria:

- A. actinomycosis;
- B. Tuberculosis;
- B. Deep mycoses;
- G. Leprosy.

6. Try mantoux put for:

- A. Selection of persons to be revaccination;
- B. Therapeutic goals;

- C. Prevention tuberculosis;
G. Control efficiency treatment.
- 7.** What kind drugs use for specific prevention tuberculosis?
A) ZhKSV-E;
B. BCG-M;
B. DPT;
G. BCG.
- 8.** At diagnostics diphtheria do sowing researched material on Wednesday:
A) RU;
B. Endo;
C. Levin;
G. Clauberg;
D. Ploskirev.
- 9.** Factors virulence pathogen tuberculosis are:
A. Capsule;
B. Cord factor;
C. Endotoxin;
G. Exotoxin;
D. Lipids cellular walls.
- 10.** What kind methods "enrichment » apply at microscopic diagnosticstuberculosis?
A. Homogenization and precipitation;
B. Price method;
B. Method flotation;
G. Method deep cultivation.
- 11.** Factors pathogenicity pathogen whooping cough
A. Filamentous hemagglutinin;
B. whooping cough toxin;
B. Extracellular adenylate cyclase;
G. Endotoxin.
- 12.** Property pathogen whooping cough
A. Demanding on nutrient media;
B. Biochemically few active;
B. Highly sensitive to environmental factors;
G. growing on simple environments.
- 13.** On what environments growing pertussis causative agent?
A. MPA;
B. Caseinovo - coal agar;
C. Wednesday Clauberg;
G. Wednesday Borde-Jangu.
- 14.** What kind epidemiological peculiarities characteristic for leprosy?
A. The source is a sick person;
B. Contact way transfers;
B. Airborne transmission;
G. A source - rodents.
- 15.** What principles underlie the clinical and immunological classificationleprosy?
A. Histological manifestations;
B. Bacterioscopic data;
B. Results of a skin-allergic test;
G. bacteriological data.
- 16.** What kind methods allow distinguish pathogens tuberculosis from pathogen leprosyat carrying out microbiological diagnostics?
A) Coloring on Tsil-Nelsen;
B. Growth on artificial nutrient media;
C. Statement of skin-allergic samples;
D. Definition pathogenicity for maritime pigs and rabbits.

17. Methods microbiological diagnostics plague:

- A. Bacteriological;
- B. Bacterioscopic;
- C. Biological;
- G. Serological.

18. Methods differentiation species brucella:

- A. Need in CO₂;
- B. Tinctorial properties;
- B. Bacteriostatic action of cells;
- G. Antigenic structure.

19. Make logical pairs: question-answer
Susceptible animals:

- | | |
|--------------------|----------------|
| 1. M. Bovis | A. Marine pigs |
| 2. M. leprae | B. rabbits |
| 3. M. tuberculosis | B. armadillos |

21. Make up brain teaser couples: question answer

- | | |
|-----------------|--------------------|
| 1. M. leprae | A. Leprosy |
| 2. M. kansasii | B. Mycobacteriosis |
| 3. M. africanum | B. Tuberculosis |
| 4. M. Avium | |

21. Compose brain teaser couples:

Main methods microbiological diagnostics

- | | |
|--------------------|---------------------|
| A) Bacterioscopic | 1. Tularemia; |
| B. bacteriological | 2. Siberian ulcer; |
| C. Serological | 3. Both. |
| G. Biological | 4. Neither other.D. |
| Allergological | |

"THE CAUSES OF DIPHTHERIA, WHOOPING COUGH, PARACROUP, TUBERCULOSIS, ZOONOUS INFECTIONS»

II OPTION

(Choose one or some correct answers)

1. Infection people brucellosis happens:

- A. In contact with sick animals;
- B. Through milk and dairy products;
- C. Through postpartum discharge;
- G. At contact With sick people.

2. For productions samples Burne apply:

- A) Pestin;
- B. Antraksin;
- C. Tulyarin;
- G. Brucellin.

3. As in a clean culture located diphtheria sticks?

- A. Disorderly;
- B. Location cells in form chains;
- C. Location cells in form "palisade";
- G. Location of cells in form V, x.

4. Ways transmission diphtheria:

- A. Airborne;
- B. Contact;
- B. Alimentary;
- G. Transmissible.

5. Which material for microbiological research should be taken from sickat suspicion on diphtheria?
- A. Mucus from the pharynx;
 - B. Film from the pharynx;
 - C. Slime from nose
 - D. Blood.
6. Nutrients environments for cultivation pathogen diphtheria:
- A. MPA;
 - B. Tellurite blood agar;C. Yolk-salt agar;
 - G. Rolled up serum.
7. How conditioned acid resistance mycobacteria?
- A. A large amount of peptidoglycan;
 - B. The presence tuberculin;
 - B. The presence of LPS in the outer membrane;
 - G. Mikolov acids.
8. For cultivation M. leprae carry out:
- A. Blood agar culture;
 - B. Infection armadillos;
 - B. Infection of a rabbit in testis;
 - G. Infection clothes lice.
- 9.characteristic location pathogen leprosy in affected fabrics:
- A. In intercellular spaces;
 - B. Intracellularly;
 - C. AT form long chains;
 - G. Forms clusters cells in form balls.
10. Method accelerated bacteriological diagnostics tuberculosis:
- A) Homogenization;
 - B. Microcultivation;
 - C. precipitation;
 - D. Method Price.
11. What kind cultural properties characteristic for M.tuberculosis?
- A. Dry colonies with uneven edges;
 - B. R-shapes;
 - C. Delicate wrinkled film on the surface of the liquid nutrientenvironment;
 - G. Smooth even colonies white or gray colors.
12. Which antigen use pi staging reactions Mitsuda?
- A) autoclaved suspension pathogen leprosy, received throughhomogenization content leprosy;
 - B. Lepromin-A;
 - B. Integral lepromin;
 - G. Dry purified tuberculin.
13. Planned specific diphtheria prophylaxis postponed until 3-4monthly age child in connections With:
- A) The receipt of secretory Ig A With milk mothers;
 - B. Lack of formed normal microflora;C. Working out high credits own antibodies;
 - D. Availability Ig g, received from mothers through placenta
14. For treatment chronic forms what zoonotic infections apply killedvaccines?
- A) Plague;
 - B. Tularemia;
 - B. Anthrax;G. Brucellosis.
15. bacteria virulent in R-shape:
- A) Brucella;
 - B. Anthrax bacilli;C. Francisella;

G. Yersinia.

16. Most often occurring pathogens brucellosis:

A. V. melitensis; B.

b. ovis;

C. b. abortus;

G. b. neotome.

17. Factors Brucella pathogenicity:

A. Endotoxin;

B. Exotoxin;

B. Enzymes of aggression;

G. Capsule.

18. For serodiagnosis brucellosis apply:

A. Reaction Wright;

B. Opson-phagocytic reaction;

C. reaction Heddleson;

G. RPGA.

19. Make up brain teaser couples: question answer

1. Biovar gravis A. Forms large smooth red colonies

2. Biovar mitis B. forms small black colonies

C. Forms large rough gray colonies.

20. Make up brain teaser couples: question answer

1. M. Bovis A. Marine pigs;

2. M. leprae B. Rabbits;

3. M. tuberculosis B. Battleships.

21. Make up brain teaser couples: question answer

1. Allocate very many pathogens

2. Allocate Little pathogens

3. More dangerous for surrounding

4. Can to be source infections at diphtheria

A) Sick diphtheria;

B. Bacteriocarriers pathogens diphtheria; C. Both;

G. Neither neither other

"THE CAUSES OF DIPHTHERIA, WHOOPING COUGH, PARACROUP, TUBERCULOSIS, ZOONOUS INFECTIONS»

II OPTION

(Choose one or some correct answers)

1. For cultivation pathogens tuberculosis use nutritious environments:

A. Levenshtein-Jensen;

B. Levin;

C. Petragnani;

G. Clauberg.

2. characteristic location diphtheria sticks in clean culture:

A) bunches;

B. AT form chains;

C. AT form "palisade";

G. Under angle to each other friend.

3. Antidiphtheria antitoxic serum applied for:

A. Emergency prevention;

B. Planned prevention;

- C. treatment;
- G. Performances skin - allergic test.
- 4.** Main tests, used for identification diphtheria sticks:
 - A. Test for cystinase;
 - B. samples to indole;
 - C. Try on urease;
 - G. Try on H₂S.
- 5.** Properties pathogen whooping cough
 - A. Gram-negative rod;
 - B. Forms exotoxin;
 - B. Biochemical is not very active;
 - G. Forms disputes.
- 6.** On what kind bodies renders pathological action diphtheria toxin?
 - A. Cardiac muscle;
 - B. kidneys;
 - C. Adrenals;
 - G. Nervous ganglia.
- 7.** On what environments can cultivate pathogen diphtheria?
 - A. MPA;
 - B. Tellurite blood agar;
 - C. Yolk-salt agar;
 - G. Curled serum .
- 8.** What properties possesses pathogen whooping cough?
 - A. Demanding on nutrient media;
 - B. Biochemical few active;
 - B. Highly sensitive to environmental factors;
 - G. growing on simple environments.
- 9.** Ways transmission pathogen leprosy:
 - A. Airborne;
 - B. Sexual;
 - C. Contact;
 - G. Transmissive.
- 10.** Prevention tuberculosis held introduction:
 - A. Anatoxin;
 - B. Antitoxin;
 - V. Tuberculin;
 - G. BCG.
- 11.** Biological models for cultivation pathogen leprosy:
 - A. Guinea pigs;
 - B. rabbits;
 - B. Golden hamsters;
 - G. Battleships.
- 12.** Methods "enrichment" researched material at microscopicdiagnostics tuberculosis:
 - A. Homogenization and precipitation;
 - B. Price method;
 - B. Flotation method;G. PCR.
- 13.** For prevention leprosy apply:
 - A. Dry purified tuberculin;
 - B. Integral lepromin;
 - B. DPT;
 - G. BCG.
- 14.** What kind epidemiological peculiarities characteristic for leprosy?
 - A. The source is a sick person;
 - B. Contact way transfers;
 - B. Airborne transmission;

G. A source - rodents.

15. Vaccine, applied for prevention brucellosis:

A. STI;

B. Living corpuscular Elbert-Gaisky;

C. EV;

G. live corpuscular Vershilova (VA-19A).

16. Material from sick for bacteriological research at tularemia:

A) Blood;

B. Puncture of lymph nodes;

C. Sputum;

G. Serum blood.

17. Factors pathogenicity of anthrax bacilli:

A) Drank;

B. disputes;

B. Endotoxin;

G. Exotoxin.

18. Test "pearl necklaces» on environment With penicillin apply for identification:

A) Yersinia;

B. Francisella;

C. Brucella;

G. Anthrax bacilli

19. Make up brain teaser couples: question answer

1. b. Pertussis A. paraptussis;

2. L. Pneumophila b. Whooping cough;

3. b. Paraptussis B. Paratyphoid;

G. Legionellosis.

20. Make logical pairs: question-answer Susceptible animals:

1. M. Bovis A. Marine pigs

2. M. leprae B. rabbits

3. M. tuberculosis B. armadillos

21. Morphological and tinctorial properties pathogens blood infections:

1. Gram positive sticks A. Yersinia plague

2. Gram negative sticks B. Pathogen tularemia

3. Ovoid form B. Both

4. Forms subterminal controversy G. Neither then, nothing else

**"THE ACTIVITIES ANAEROBIC CLOSTRIDIAL INFECTIONS.
PATHOGENIC SPIROCHETES AND SPIROCHETES. MYCOPLASMAS. CHLAMYDIA" I OPTION**

(Choose one or some correct answers)

1. The onset of tetanus is caused by the ingestion of body:

A) Brucella melitensis;

B. Exotoxins Clostridium difficile;

B. Clostridium tetani and its exotoxin;

G. Clostridium novyi through wound.

2. By type breathing clostridia:

A) obligate anaerobes;

B. Optional anaerobes;

C. obligate aerobes;

D. Facultative aerobes;

D. Microaerophiles.

3. To pathogens gas gangrene relate:

- A. *Clostridium perfringens*;B.
- Clostridium tetani*;
- C. *Clostridium botulinum*;G.
- Clostridium novyi*.

4. Ways transmission botulism:

- A. Parenteral;
- B. Wound;
- B. Contact household;
- G. Food.

5. pathogens tetanus are:

- A. *Fusobacteria*;
- B. *Clostridia*;
- C. *Bacteroids*;
- G. *Peptococci*.

6. For clostridia characteristic:

- A. Capsule formation;
- B. spore formation;
- C. Presence of volutin grains;
- G. Anaerobic type breathing.

7. Immunity after transferred botulism:

- A) Antitoxic;
- B. Antibacterial;
- C. Local;
- G. Not is being formed.

8. Non-spore-forming anaerobes:

- A) *Bacteroids*;
- B. *Clostridia*;
- B. *Fusobacteria*;
- G. *Waylonelles*.

9. Immunobiological drugs for prevention and treatment botulism:

- A. Antitoxic serum;
- B. DPT;
- B. Tetraanatoxin;
- G. ADS.

10. Pathogen syphilis:

- A. *T. pertenuis*;
- B. *T. pallidum*;
- B. *N. gonorrhoeae*;
- G. *N. meningitidis*.

11. Describe pathogen leptospirosis:

- A. Thin light threads with curved ends;
- B. Are stained in purple Colour;
- B. Number of curls 20-40;G.
- form cysts.

12. Sustainability pathogen syphilis in environmental environment:

- A. Resistant to disinfectants;
- B. Weakly resistant to the environment;
- C. sustainable to elevated temperature;
- G. sustainable to drying out.

13. For syphilis characteristic:

- A. Penetration of the pathogen through skin and mucous membranes;
- B. Transplacental infection by;
- C. leaking cyclically;
- G. Leaks in form sepsis.

14. What properties have spirochetes?

- A) Have thin cellular wall;
- B. Gram-negative;
- B. Thin spirally curved cells;
- G. Have cytoplasmic cylinder.

15. What kind properties characteristic for chlamydia?

- A. Gram-negative;
- B. Prokaryotes;
- B. Obligate intracellular parasites;
- G. Have twisted shape.

16. A source infections at loose typhus:

- A) Sick;
- B. Carrier;
- B. Animals;
- G. Lice.

17. Immunity at loose typhus:

- A. Antibacterial;
- B. Antitoxic;
- C. non-sterile;
- G. Local.

18. Methods cultivation rickettsia:

- A. On blood agar;
- B. AT anaerostat;
- V. V chicken embryo;
- G. On serum environments.

19. Make up brain teaser couples:

- A) bioassay on suckling rabbits
- B. Aniline dyes are well perceivedC. Microscoping in dark field vision

1. pathogens disease Lyme
2. Pathogen leptospirosis
3. Both
4. Neither then, neither other.

20. Immunological drugs for creation active immunity:

- | | |
|-------------------------|-----------------------|
| A. DTP | 1. Tetanus |
| B. Tetanus toxoid serum | 2. Gas gangreneV. |
| ADS-m | 3. Both |
| G. Antigangrenous serum | 4. Neither not other. |

21. Make up brain teaser couples: question answer

- A) For bacterioscopic diagnostics use microscopy in dark field.
- B. For bacterioscopic diagnostics carry out microscopy smears, painted onGram.
- B. For diagnosis, a skin-allergic test is performed.G. Available contact household transmission path.
- D. Anthroponosis.

1. Syphilis
2. Gonorrhea
3. Both
4. Neither then, neither other

"THE ACTIVITIES ANAEROBIC CLOSTRIDIAL INFECTIONS.
PATHOGENIC SPIROCHETES AND SPIROCHETOSIS. MYCOPLASMAS. CHLAMYDIA"II OPTION

(Choose one or some correct answers)

1. To pathogens anaerobic infections applies to:

- A. Clostridia;
- B. Mycoplasmas;

- B. Mycobacteria;
- G. Chlamydia.
- 2. Disease botulism conditioned hit in organism person:**
 - A) Brucella bovis;
 - B. Clostridium exotoxins tetani;
 - C. Clostridium botulinum and their exotoxins;
 - G. Dispute Clostridium difficile.
- 3. Clostridia form:**
 - A. Hemozolin;
 - B. catalase;
 - B. Lecithinase;G.
 - DNA azu.
- 4. Clostridial anaerobes cultivated in environment:**
 - A. Wilson-Blair;
 - B. Salt environment;
 - C. biliary bouillon;
 - G. Tall column sugar agar.
- 5. For diagnostics gas gangrene apply:**
 - A. Bacteriological research method;
 - B. Biological;
 - C. Microscopic;
 - G. Serological method.
- 6. Tetanus - this is infection:**
 - A) anaerobic;
 - B. Contact;
 - C. Intestinal;
 - G. Wound.
- 7. Clostridia - this is:**
 - A. Gram + aerobes;
 - B. Gram- aerobes;
 - V. Gram+ anaerobes;
 - G. Gram- anaerobes.
- 8. name peculiarities spirochete:**
 - A) Gram negative bacteria;
 - B. Have a motor fibrillar apparatus;
 - C. Have twisted shape.
 - G. Are absolute parasites.
- 9. Peculiarities Borrelia:**
 - A) Collection bacteria With 3-8 curls
 - B. Thin tortuous cells With bent ends
 - B. Stained according to Romanovsky-Giemsa in purple
 - G. Weak perceive aniline dyes.
- 10. Morphology pathogen syphilis:**
 - A. A thin bacterium of a spiral shape;
 - B. thick wand;
 - B. Bean-shaped cocci;
 - G. Vibrio.
- 11. Cultural properties pathogen syphilis:**
 - Can i cultivate in testicle a rabbit;
 - B. Can cultivate on environments containing pieces organs;
 - C. Cultivated in anaerobic conditions;
 - G. Cultivate can in aerobic conditions.
- 12. Methods bacterioscopic diagnostics syphilis:**
 - A) Coloring silvering;
 - B. Methylene blue stain;
 - B. Dark field microscopy;
 - G. coloring by Gram.

13. name obligate intracellular parasites:

- A) Rickettsia;
- B. Actinomycetes;C.
- Spirochetes;
- G. Chlamydia.

14. Serological reactions used at diagnostics loose typhus:

- A) Agglutination;
- B. RSK;
- C. RPGA;
- G. precipitation.

15. Mycoplasmas cause:

- A) Atypical pneumonia;
- B. Lesions of the genitourinary tract;
- C. Sypnoy typhus;
- G. Returnable typhoid.

16. Main method identifying chlamydia is:

- A. Coloring according to Romanovsky-Giemsa;
- B. coloring by Neisser;
- B. Coloring according to Zdradovsky;
- G. coloring by Burri.

17. name main factors pathogenicity rickettsia:

- A. Microcapsule;
- B. Phospholipase A2;
- C. Adhesins (OmpA, OmpB);
- G. Exotoxin.

18. Check pathogens defiant disease respiratory tract, at which source infections is a person:

- A. C. trachomatis;
- B. M. pneumoniae;
- C. C. psittaci;
- G. C. pneumoniae.

19. Make up brain teaser couples: question answer

- A) Are transmitted airborne throughB. Are transmitted sexual through
- C. Have lipid antigen, identical lipid extract bullish heartsG. At hit in phagocytes cause unfinished phagocytosis

- 1. T. pallidum
- 2.N.gonorrhoeae
- 3. Both
- 4. Neither then, neither other

20. Make logical pairs: question answer Morphological and tinctorial

- properties of pathogens:A. Gram-positive sticks 1. Pathogen tetanus
- B. Terminal controversy 2. pathogens gas gangreneB.
- Subterminal controversy 3. Both
- G. Arranged in a chain of 4. Neither then, nothing else

21. Make up brain teaser couples: question answer

- A. Epidemic relapsing typhoid 1. b. burgdorferi
- B. syphilis 2. L. interrogans
- B. Illness Lyme 3. b. recurrentis
- D. Leptospirosis 4. T. pallidum

"THE ACTIVITIES ANAEROBIC CLOSTRIDIAL INFECTIONS.
PATHOGENIC SPIROCHETES AND SPIROCHETOSIS. MYCOPLASMAS. CHLAMYDIA" III OPTION

(Choose one or some correct answers)

1. pathogens tetanus are:
 - A. Fusobacteria;
 - B. Clostridia;
 - C. Bacteroids;
 - G. Peptococci.
2. For clostridia characteristic:
 - A. Capsule formation;
 - B. spore formation;
 - C. Presence of volutin grains;
 - G. Anaerobic type breathing.
3. Methods microbiological diagnostics botulism:
 - A. Bacterioscopic;
 - B. Bacteriological;
 - C. Biological;
 - G. Serological.
4. Clostridium perfringens is pathogen:
 - A. Food poisoning;
 - B. Pseudomembranous colitis;
 - C. Gas gangrene;
 - G. Toxinemic infections.
5. Non-spore-forming anaerobes:
 - A) Bacteroids;
 - B. Clostridia;
 - B. Fusobacteria;
 - G. Waylonelles.
6. Immunobiological drugs for prevention and treatment botulism:
 - A. Antitoxic serum;
 - B. DPT;
 - B. Tetraanatoxin;
 - G. ADS.
7. The onset of tetanus is caused by the ingestion of body:
 - A) Brucella melitensis;
 - B. Exotoxins Clostridium difficile;
 - B. Clostridium tetani and its exotoxin;
 - G. Clostridium novyi through wound.
8. By type breathing clostridia:
 - A) obligate anaerobes;
 - B. Optional anaerobes;
 - B. Obligate aerobes;
 - G. Microaerophiles.
9. To pathogens gas gangrene relate:
 - A. Clostridium perfringens;
 - B. Clostridium tetani;
 - C. Clostridium botulinum; G.
 - Clostridium novyi.
10. Ways transmission botulism:
 - A. Parenteral;
 - B. Wound;
 - B. Contact household;
 - G. Food.
11. For syphilis characteristic:
 - A. Penetration of the pathogen through skin and mucous membranes;

- B. transplacental infection by;
- C. leaking cyclically;
- G. Leaks in form sepsis.

12. Antigens used for productions RSK at diagnostics syphilis:

- A) O antigen;
- B. Cardiolipin;
- C. Soluble antigen;
- G. Treponemal specific.

13. What properties have spirochetes?

- A) Have thin cellular wall;
- B. Gram-negative;
- B. Thin spirally curved cells;
- G. Have cytoplasmic cylinder.

14. What kind properties characteristic for chlamydia?

- A. Gram-negative;
- B. Prokaryotes;
- B. Obligate intracellular parasites;
- G. Have twisted form.

15. Describe pathogen leptospirosis:

- A. Thin light threads with curved ends;B. Are stained in purple Colour;
- B. Number of curls 20-40;G. form cysts.

16. A source infections at loose typhus:

- A) Sick;
- B. Carrier;
- B. Animals;
- G. Lice.

17. Immunity at loose typhus:

- A. Antibacterial;
- B. Antitoxic;
- C. non-sterile;
- G. Local.

18. Methods cultivation rickettsia:

- A. On blood agar;
- B. AT anaerostat;
- V. V chicken embryo;
- G. On serum environments.

19. Make up brain teaser couples: question answer

- A) For bacterioscopic diagnostics use microscopy in dark field.
- B. For bacterioscopic diagnosis, microscopy of smears stained according to Gram.
- B. For diagnosis, a skin-allergic test is performed.G. Available contact household transmission path.
- D. Anthroponosis.

- 1. Syphilis
- 2. Gonorrhea
- 3. Both
- 4. Neither then, neither other

20. Immunobiological drugs for creation active immunity:

- | | |
|--------------------------|------------------------------------|
| A. DTP | 1. Tetanus |
| B. Tetanus toxoid serum | 2. Gas gangreneV. |
| ADS-M | 3. Both |
| G. Antigangrenous serum. | four. Neither then, neither other. |

21. Install conformity called infections and kind pathogen:

- | | |
|----------------------|------------------|
| A) Tetanus | 1. Cl. botulinum |
| B. Gas gangrene | 2. Cl. tetani |
| B. Botulism | 3. F. nucleatum |
| G. Fusospirochetosis | 4. Cl. novyi |

**"THE ACTIVITIES ANAEROBIC CLOSTRIDIAL INFECTIONS.
PATHOGENIC SPIROCHETES AND SPIROCHETOSIS. MYCOPLASMAS. CHLAMYDIA" IV OPTION**

(Choose one or some correct answers)

- 1. Tetanus - this is infection:**
 - A) anaerobic;
 - B. Contact;
 - C. Intestinal;
 - G. Wound.
- 2. To pathogens anaerobic infections applies to:**
 - A. Clostridia;
 - B. Mycoplasmas;
 - B. Mycobacteria;
 - G. Chlamydia.
- 3. Clostridia - this is:**
 - A. Gram + aerobes;
 - B. Gram- aerobes;
 - V. Gram+ anaerobes;
 - G. Gram- anaerobes.
- 4. For diagnostics gas gangrene apply:**
 - A. Bacteriological research method;
 - B. Biological;
 - C. Microscopic;
 - G. Serological method.
- 5. Disease botulism conditioned hit in organism person:**
 - A) Brucella bovis;
 - B. Clostridium exotoxins tetani;
 - C. Clostridium botulinum and their exotoxins;
 - G. Dispute Clostridium difficile.
- 6. Clostridia form:**
 - A. Hemolysin;
 - B. catalase;
 - B. Lecithinase;G.
 - DNA azu.
- 7. Clostridial anaerobes cultivated in environment:**
 - A. Wilson-Blair;
 - B. Salt environment;
 - C. biliary bouillon;
 - G. Tall column sugar agar.
- 8. name peculiarities spirochete:**
 - A) Gram negative bacteria;
 - B. Have a motor fibrillar apparatus;
 - C. Have twisted shape.
 - G. Are absolute parasites.
- 9. Morphology pathogen syphilis:**
 - A. A thin bacterium of a spiral shape;
 - B. thick wand;
 - B. Bean-shaped cocci;G. Vibrio.
- 10. Peculiarities Borrelia:**
 - A) Collection bacteria With 3-8 curls
 - B. Thin tortuous cells With bent ends
 - B. Stained according to Romanovsky-Giemsa in purpleG. Weak

perceive aniline dyes.

11. Methods bacterioscopic diagnostics syphilis:

- A) Coloring silvering;
- B. Methylene blue stain;
- B. Dark field microscopy;
- G. coloring by Gram.

12. Cultural properties pathogen syphilis:

- Can i cultivate in testicle a rabbit;
- B. May be cultured on media containing organ pieces;
- C. Cultivated in anaerobic conditions;
- G. Cultivate can in aerobic conditions.

13. name obligate intracellular parasites:

- A) Rickettsia;
- B. Actinomycetes;C.
- Spirochetes;
- G. Chlamydia.

14. Serological reactions, used at diagnostics loose typhus:

- A) Agglutination;
- B. RSK;
- C. RPGA;
- G. precipitation.

15. Mycoplasmas cause:

- A) Atypical pneumonia;
- B. Lesions of the genitourinary tract;
- C. Synov typhus;
- G. Returnable typhoid.

16. name main factors pathogenicity rickettsia:

- A. Microcapsule;
- B. Phospholipase A2;
- C. Adhesins (OmpA, OmpB);
- G. Exotoxin.

17. Check pathogens defiant disease respiratory tract, at which source infections is a person:

- A. C. trachomatis;
- B. M. pneumoniae;
- C. C. psittaci;
- G. C. pneumoniae.

18. Main method identifying chlamydia is:

- A. Coloring according to Romanovsky-Giemsa;
- B. coloring by Neisser;
- B. Coloring according to Zdradovsky;
- G. coloring by Burri.

19. Make up brain teaser couples: question answer

- A. Epidemic relapsing typhoid
- B. syphilis
- B. Illness Lyme
- D. Leptospirosis

- 1. b. burgdorferi
- 2. L. interrogans
- 3. b. recurrentis
- 4. T. pallidum

20. Make up brain teaser couples: question answer

- A) Gram negative
- B. Kokki
- C. Sticks
- G. form subterminal controversy

- 1. Bacteroids
- 2. Waylonelles
- 3. Both
- 4. Neither then, neither other.

21. Make up brain teaser couples: question answer

- A. Gr+Kokki
- B. Gr+ sticks
- B. Aerobes
- D. Anaerobes

- 1. Peptostreptococci
- 2. Clostridia
- 3. Both
- 4. Neither then, neither other.

«PRIVATE VIROLOGY. PATHOGENIC FUNGI»I OPTION

(Choose one or some correct answers)

1. In pathogenesis viral diseases decisive plays a role:
 - a) the virulence of the virus;
 - b) toxigenicity of the virus;
 - in) level lysozyme;
 - D) reaction organism on cells, affected virus.
2. Install serological type virus influenza may with help:
 - a) agglutination reactions on glass;
 - b) reactions braking hemagglutination;
 - c) reactions indirect hemagglutination;
 - D) reactions hemagglutination.
3. In pathogenesis AIDS important place occupies:
 - a) transformation of PrP^c proteins into PrP^{sc} proteins;
 - b) unrestrained proliferation B-lymphocytes;
 - c) accumulation pathological myeloma proteins;
 - D) defeat T-helpers and macrophages.
4. Interferon provides antiviral protection cells, because prevents:
 - a) virus adsorption on cage;
 - b) penetration virus in cell;
 - c) reproductions virus;
 - D) lysis affected cells;
5. HIV refers to virus group:
 - a) DNA-genomic;
 - b) RNA-genomic;
 - c) complex;
 - D) simple.
6. For serodiagnosis viral hepatitis apply:
 - a) hemagglutination inhibition reaction;
 - b) enzyme immunoassay analysis;
 - c) reaction of indirect (passive) hemagglutination;
 - D) reaction hemagglutination;
7. Neurotropic viruses are considered:
 - a) a virus flu;
 - b) hepatitis C virus;
 - c) rabies virus;
 - D) virus rubella.
8. Virus Epstein-Barr calls:
 - a) Sarcoma Kaposi;
 - b) Infectious mononucleosis;
 - c) Shingles lichen;
 - D) Cytomegaly.
9. For planned specific prevention polyemylite use:
 - a) Sabin live vaccine;
 - b) toxoid;
 - c) killed vaccine;
 - D) specific serum;
10. Virus rubella calls:
 - a) Panencephalitis;
 - b) acute respiratory infection;

- c) congenital pathology;
 - D) sharp intestinal infection.
11. Virus avian influenza applies to:
 - a) to the influenza virus type C;
 - b) to the influenza virus type A;
 - c) to the influenza virus type B;
 - D) to virus influenza type D.
 12. Viruses poliomyelitis refer to family:
 - a) caliciviruses;
 - b) retroviruses;
 - c) poxviruses;
 - D) picornaviruses.
 13. Basic way transmission virus hepatitis A AND:
 - a) parenteral;
 - b) airborne;in) fecal-oral; D)
 - contact.
 14. Which type nucleic acids contains a virus hepatitis A AT?
 - a) RNA;
 - b) DNA;
 - c) DNA and RNA.
 15. What is yourself mycelium mushrooms?
 - a) it is a cell devoid of a cytoplasmic membrane;
 - b) this is totality hyphae;
 - c) it is a collection of chlamydospores;
 - d) it is multi-core structure
 16. Yeast-like mushrooms are characterized by:
 - a) presence round or oval cells;
 - b) ability multiply sexual by;
 - c) ability multiply only asexual by;
 - D) ability form disputes.
 17. Mushrooms kind Candida
 - a) relate to yeast-like mushrooms;
 - b) relate to filamentous mushrooms;
 - c) belong to filamentous fungi;
 - D) are pathogenic.
 18. At keratomycosis are affected:
 - a) the stratum corneum of the epidermis;
 - b) bones;
 - c) hair;
 - D) internal organs.

Make up brain teaser couples: question answer

19.

1. Conditionally pathogenic mushrooms: a. Trichophyton
2. Dermatophytes b. Genus Aspergillus
3. form conidia: c. Both
4. form aflatoxins: d. Neither then, neither other

20. Specify conformity between through transmission virus and disease

1. fecal-oral a. Hepatitis B
2. Parenteral b. Polio
3. airborne in. Hepatitis AND

21.

1. To rhabdoviruses relate:
2. To orthomyxoviruses relate: A. mumps virus. B. Virus rabies
B. Tick-borne encephalitis virus; G.
Viruses flu.

«PRIVATE VIROLOGY. PATHOGENIC FUNGI»

II OPTION

(Choose one or some correct answers)

1. Determine the antibodies in the patient's blood to a specific serotype virus influenza can With help:
 - a) agglutination reactions on glass;
 - b) reactions hemagglutination;
 - c) enzyme immunoassay analysis.
2. Reaction braking hemagglutination maybe to be applied for:
 - a) detection virus influenza in researched material;
 - b) identification virus flu;
 - c) definition quantities virus in the study material; D) detection antibodies to virus in blood.
3. Specify virus hepatitis A, demanding for replication participation helper virus:
 - a) VGA;
 - b) VGB;
 - c) VGC;
 - D) VGD.
4. Interferon has next action:
 - a) lysing in relation affected cells;
 - b) stimulating phagocytosis;
 - c) inhibitory broadcast;
 - D) specific binding With virus.
5. Virus influenza applies to the group of viruses:
 - a) DNA-genomic;
 - b) RNA-genomic;
 - c) complex;
 - D) families orthomyxoviruses.
6. characteristic signs families retroviruses are:
 - a) H and N antigens capsid;
 - b) enzyme reverse transcriptase;
 - c) fragmentation genome;
 - D) two identical threads RNA in genome.
7. Enterotropic are considered:
 - a) virus poliomyelitis;
 - b) hepatitis C virus;
 - c) virus rabies;
 - D) viruses coxsackie and Echo.
8. Viruses flu - this is:
 - a) DNA-containing viruses
 - b) simple viruses
 - c) RNA-containing viruses
 - D) complex viruses.
9. For specific prevention rabies use:

- a) a live vaccine;
 - b) toxoid;
 - in) inactivated vaccine;
 - D) gamma globulin.
- 10.** Antigenic drift and shift are related to the following virus antigensflu:
- a) ribonucleoprotein NP;
 - b) matrix squirrel M;
 - c) neuraminidase N;
 - D) hemagglutinin N.
- 11.** Which type—well—gluic acid contains virus wind smallpox?
- a) RNA;
 - b) DNA;
 - c) DNA and RNA;
 - D) not contains nucleic acid.
- 12.** Polio viruses are:
- a) DNA-containing viruses;
 - b) simple viruses;
 - c) RNA-containing viruses;
 - D) complex viruses.
- 13.** Which type nucleic acid contain viruses hepatitis AND and E?
- a) DNA;
 - b) RNA;
 - c) DNA and RNA;
 - D) not contains nucleic acid.
- 14.** To systemic, or deep mycoses applies to:
- a) Histoplasmosis;
 - b) Favus (scab);
 - c) Sporotrichosis;
 - D) Microsporia.
- 15.** What such conidia?
- a) Endospores;
 - b) Exospores;
 - in) spore-forming structures;
 - D) transverse partition in hyphae.
- 16.** Opportunistic mycoses:
- a) Cause pathogenic mushrooms;
 - b) Cause conditionally pathogenic mushrooms;
 - c) Cause unclassified pathogenic fungi;
 - D) Cause dermatophytes.
- 17.** Mycoses - this is diseases, caused by:
- a) bacteriab)
 - mushrooms;
 - c) the simplest;
 - D) Chlamydia.
- 18.** For allocation mushrooms from pathological material use:
- a) MPA;
 - b) Wednesday Saburo;
 - c) serum agar;
 - D) MPB.

Make up brain teaser couples: question answer

19.

- 1.** Keratomycosis: A. microsporum

- | | |
|-------------------------|------------------------|
| 2. subcutaneous mycoses | b. Pathogen versicolor |
| 3. Deep mycoses | B. Sporotrichosis |
| 4. Epidermophytosis: | G. Blastomycosis. |

20. Install conformity between through infections and view virus hepatitis A

- | | |
|---------------|----------|
| 1. fecal-oral | A) VGA |
| 2. Parenteral | B. VG AT |
| 3. Sexual | C. VGE |

21. Which type nucleic acids contain viruses?

1. Herpesviruses
2. Virus parainfluenza
3. Polio virusA) DNA
- B. RNA
- C. DNA and RNA
- G. neither then, neither other.

«PRIVATE VIROLOGY. PATHOGENIC FUNGI»

III OPTION

(Choose one or some correct answers)

1. specific factors protection organism from viruses are:
 - a) NK - cells (normal killers);
 - b) interferons;
 - c) slgA;
 - D) CD8- cells (T-killers).
2. HIV is cultivated in: a)
chicken embryo; b) culture
cells PE4;
 - c) lungs whites mice;
 - D) CD4 culture lymphocytes.
3. Synthesis interferons encoded:
 - a) genome virus;
 - b) genes HLA;
 - c) prophage;
 - D) provirus.
4. Virus influenza cultivated in:
 - a) culture CD4 lymphocytes;
 - b) lungs whites mice;
 - c) chicken embryo;
 - D) cell culture PE4.
5. Nonspecific resistance to viruses influenza subject to availability:
 - a) lysozyme;
 - b) complement;
 - c) inhibitors;
 - D) interferons.
6. To viruses hepatitis A, having complex structure include:
 - a) VGA;
 - b) VGB;
 - c) VGC;
 - D) VGE.
7. Slow viral disease are characterized by:
 - a) incubation period continues months and years;
 - b) recurrent damage to the central nervous system and immune system;in) progressive course with lethal outcome;

- D) acute course with defeat vital important organs.
8. The AIDS clinic is defined by a number of complications caused by opportunistic agents:
- a) herpes viruses;
 - b) pathogen diphtheria;
 - c) mushrooms Candida;
 - D) mycobacteria tuberculosis.
9. For specific prevention tick-borne encephalitis use:
- a) a live vaccine;
 - b) toxoid;
 - c) a killed vaccine;
 - D) antigrippin.
10. Viruses influenza refer to family:
- a) coronaviruses;
 - b) adenoviruses;
 - c) paramyxoviruses;
 - D) orthomyxoviruses.
11. For virus natural smallpox characteristic:
- a) RNA-containing simple virus;
 - b) DNA-containing complex virus;
 - c) contains hemagglutinin;
 - D) not contains hemagglutinin.
12. What class of immunoglobulins in the blood serum of a patient with hepatitis A testifies about activity (sharpness) process?
- a) IgG;
 - b) IgA;
 - c) Ig M;
 - d) Ig E.
13. How much serotypes have viruses polio?
- a) five
 - b) 7
 - c) 3
 - D) 2
14. Virus immunodeficiency human characterized next properties:
- a) DNA-containing;
 - b) RNA-containing;
 - c) simple virus;
 - D) complicated virus.
15. What represents yourself mycelium mushrooms?
- a) it is a cell devoid of a cytoplasmic membrane;
 - b) this is totality hyphae;
 - c) it is a collection of chlamydospores;
 - d) it multicore structure.
16. Yeast-like mushrooms not are characterized by:
- a) presence round or oval cells;
 - b) ability multiply sexual by;
 - in) ability multiply only asexual by; D) ability form disputes.
17. Mushrooms kind Candida
- a) relate to yeast-like mushrooms; b) relate to filamentous mushrooms;
 - c) belong to filamentous fungi; D) are pathogenic.
18. By relation to temperature pathogenic mushrooms are:
- a) psychrophiles; b) mesophylls;
 - c) thermophiles;

D) Everybody answers correct.

Make up brain teaser couples: question answer

19.

- | | |
|--|--------------------------|
| 1. Conditionally pathogenic mushrooms a . Trichophyton | |
| 2. Dermatophytes | b. Genus Aspergillus |
| 3. form conidia: | B. Both |
| 4. form aflatoxins: | G. Neither nothing else. |

20.

Specify conformity between through transmission virus and disease

- | | |
|---------------|----------------|
| 1. fecal-oral | A. Hepatitis B |
| 2. Parenteral | B. Polio |
| 3. airborne | B. Hepatitis A |
| | G. Rubella |

21.

Which viruses discovered the following antigens?

1. HBs -antigen
2. Hemagglutinin
 - A) Virus measles
 - B. Virus hepatitis A AT
 - C. Virus poliomyelitis.

«PRIVATE VIROLOGY. PATHOGENIC FUNGI»

IV OPTION

(Choose one or some correct answers)

1. HIV refers to virus group:
 - a) DNA-genomic; b) RNA-genomic; in) complex;
 - D) families orthomyxoviruses;
2. Viruses parainfluenza - this is:
 - a) DNA-containing viruses;
 - b) simple viruses;
 - c) RNA-containing viruses;
 - D) complex viruses.
3. Interferon provides antiviral protection cells, because prevents:
 - a) reproductions virus;
 - b) lysis of the affected cell;
 - c) activation killers;
 - D) adsorption virus on cage
4. Nonspecific factors protecting the body from influenzaare:
 - a) the complement system;
 - b) inhibitors;
 - c) interferons;
 - D) slgA;
5. Broadcast HIV infections going on next ways:
 - a) parenteral;
 - b) alimentary;

- c) sexual;
 - D) airborne.
6. For serodiagnosis viral hepatitis apply:
- a) hemagglutination inhibition reaction;
 - b) enzyme immunoassay analysis;
 - c) reaction of indirect (passive) hemagglutination;
 - D) reaction hemagglutination.
7. Causative agents slow infections may to be:
- a) prions;
 - b) tick-borne encephalitis virus;
 - c) polio virus;
 - e) virus flu.
8. Enterotropic are considered:
- a) polio virus;
 - b) virus rabies;
 - c) virus hepatitis A FROM;
 - D) coxsackie viruses and Echo.
9. For planned specific prevention influenza use:
- a) a live vaccine;
 - b) toxoid;
 - c) inactivated whole virion vaccine;
 - D) antigrippin.
10. Virus measles on structure:
- a) a simple virus;
 - b) complicated virus;
 - c) It has supercapsid;
 - D) not It has supercapsid.
11. Viruses parainfluenza include:
- a) to the genus Paramyxovirus;
 - b) to kind Lyssavirus;
 - c) to the genus Pneumovirus;
 - D) to kind Morbillivirus.
12. For specific prevention poliomyelitis use:
- a) BCG;
 - b) DPT;
 - c) a live vaccine received by Smorodintsev A.A. and Chumakov M.P.;
 - D) anti-rabies vaccine.
13. Which way transmission hepatitis AT, FROM, D, G is main?
- a) fecal-oral;
 - b) parenteral;
 - c) airborne;
 - D) contact.
14. Which type nucleic acid contain viruses hepatitis AND and E?
- a) DNA;
 - b) RNA;
 - c) DNA and RNA;
 - D) not contains nucleic acid.
15. ringworm lichen called mushrooms kind:
- a) Trichophyton;
 - b) Aspergillus;
 - c) Candida
 - D) Fusarium.
16. To systemic, or deep mycoses applies to:

- a) Histoplasmosis;
- b) Favus (scab);
- c) Sporotrichosis;
- D) Microsporia.

17. What such conidia?

- a) Endospores;
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- a) cause pathogenic mushrooms;
- b) cause conditionally pathogenic mushrooms;
- c) cause unclassified pathogenic mushrooms;
- D) cause dermatophytes.

Make up brain teaser couples: question answer

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| 1. Keratomycosis: | A. microsporum |
| 2. subcutaneous mycoses | b. Pathogen versicolor |
| 3. deep mycoses | B. Sporotrichosis |
| 4. Epidermophytosis: | G. Blastomycosis |
20. Install conformity between type nucleic acids genome and view virushepatitis A

- | | |
|--------|-------|
| 1-DNA | A.VGA |
| 2- RNA | B.VGB |
| | V.VGC |
| | G.VGD |
| | D.VGE |

21. What kind reactions used when diagnostics

- 1. Polio
- 2. Hepatitis A AT
 - A) reaction neutralization color samplesB.
 - reaction indirect hemagglutination C. Both
 - G. Neither then, neither other.