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БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА МИКРОБИОЛОГИИ, ВИРУСОЛОГИИ, ИММУНОЛОГИИ

ЛАБОРАТОРНЫЙ ПРАКТИКУМ ПО ВИРУСОЛОГИИ

LABORATORY WORKBOOK IN VIROLOGY

5-е издание

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Ответственная за выпуск Т. А. Канашкова
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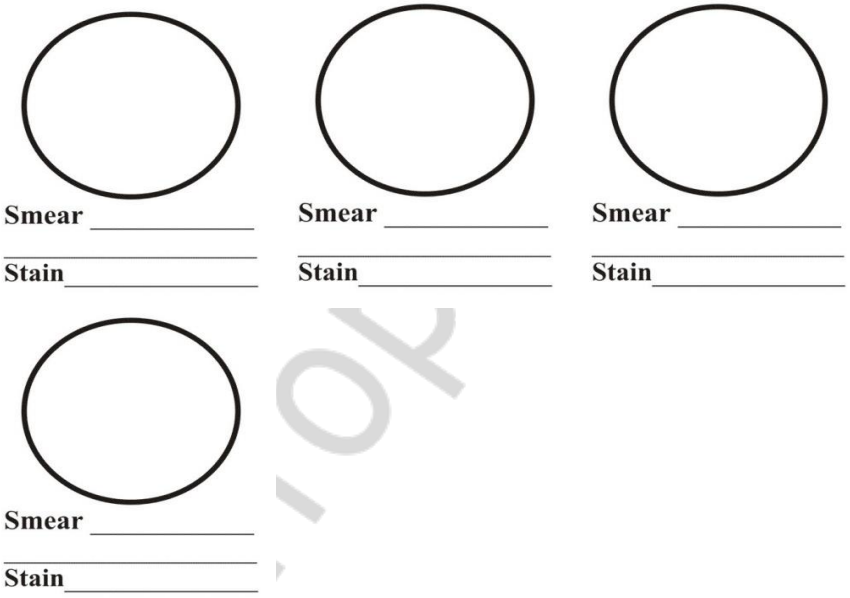
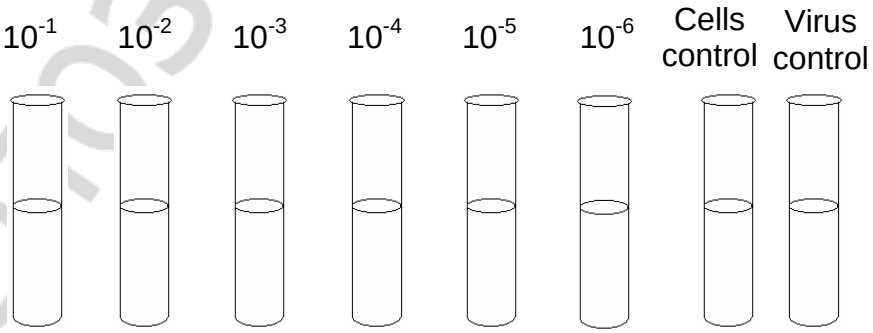
Class № 1. **Date_____**

Topic: Methods of investigations in virology. Bacteriophages.

List of questions to study:	Medical Microbiology / F. H. Kayser, K. A. Bienz, J. Eckert , R. M. Zinkernagel // Thieme New York 333, Seventh Avenue, New York, NY 10001 USA.- 2005.: 1. P. 376 – 411.
Viruses. Taxonomy and morphology of viruses. Mechanisms of reproduction. Strict parasitism and cytotropism f viruses. The types of viral infection. The mechanisms of antiviral immunity. Methods of viral infections diagnostics. Culturing of viruses in hen embryos and in laboratory animals. Methods of infection, indication and identification of viruses. Serological methods in virology. Hemagglutination inhibition test (HIT), hemadsorbtion inhibition test, neutralization test, immunoenzyme analysis (ELYSA). Molecular-genetic methods. Viruses of bacteria (bacteriophages). Virulent and moderate bacteriophages. Methods for bacteriophages titration. Use of bacteriophages in medical practice. Phagodiagnostics and phagotyping.	

Laboratory work

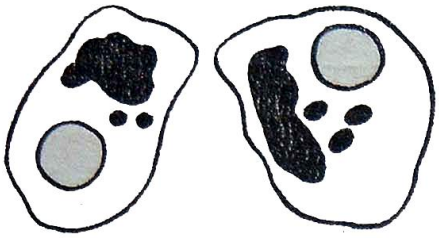
Laboratory exercises	**Laboratory report**
1. Demonstration: 1. Chicken fibroblasts, eosin; 2. Hep2 cell line, normal; 3. Cytopathic effect of adenoviruses 4. Hemadsorption test.	
2.Virus titration by color test.	 Conclusion:

4. HIT with pared sera for viral disease diagnostics. Ingredients: - sera of the patient S1 – taken at admission, S2 – taken in two weeks. - erythrocytes suspension, - standard virus dilution, - saline solution.	1/10 1/20 1/40 1/80 1/160 1/320 1/640 $Serum_1$ control Virus control 1/10 1/20 1/40 1/80 1/160 1/320 1/640 $Serum_2$ control Conclusion:

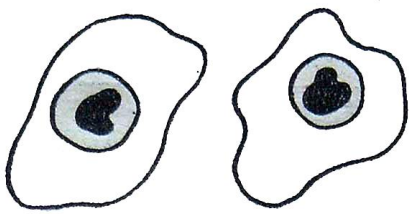
Tutor signature_____

Additional materials to class № 1

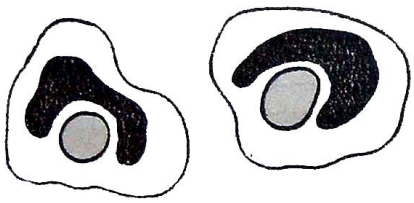
Bacteriophages titration by Gracia.	Viral inclusions
A. Prepare enough LB plates for the experiment. Plates should be prewarmed at 37 C. Б. Prepare serial dilutions of filtrate, containing the phge of interest: 1:10, 1:100, 1:1000 etc. B. Prepare top agar (0,7%) media. It must be maintained on waterbath at 45-50°C. C. Prepare suspension of phage sensitive bacteria/ D. Mix 1ml of each phage dilution with 0,1 ml of bacteria and 2,5 ml of top agar and immediately pour onto prewarmed LB plates. E. Allow plates to cool until agar has set. Invert the plates (lid side down), and incubate 12-24 hours at 37°C. D. Count plaques and record the results. Phage activity can be expressed as a titer (maximal dilution at which phages retain ability to lyse bacteria). More precisely phage activity can be calculated sa the concentration of pfu (plaque forming units) in the ml of original material. N = n (number of plaques) x dilution. Example: on plate with phage dilution 10^{-5} 15 plaques were found. N = $15x10^5$ = $1,5x10^6$ pfu/ml of initial material.	A. Viral inclusions are usually revealed by microscopy of infected cells and are the specific signs of viral infection of the cell. Certain viral inclusion has diagnostic importance. B. VI were discovered by D. Ivanovsky (abnormal crystal intracellular inclusions in affected leaves of tobacco - Ivanovsky crystals). C. VI can be revealed in nucleus and/or cytoplasm of the infected cell. D. VI may be basophilic or eosinophilic and can vary in shape, quantity and location in the cell. E. Characteristic nuclear VI can be observed in cells infected by herpesviruses, polyomaviruses, foot and mouth disease virus, adenoviruses, flaviviruses etc. F. Cytoplasmic VI are usually noted in smallpox, influenza, rabies etc.



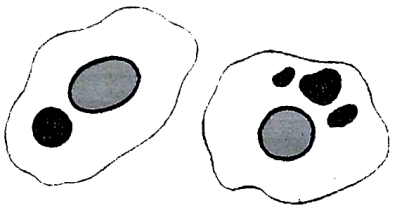
Cells infected by smallpox virus (Guarnieri bodies)



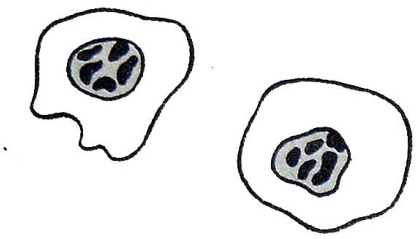
Cells infected by herpes virus (Cowdry bodies)



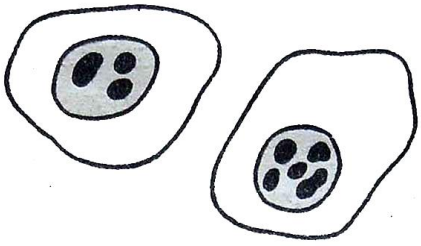
Cells infected by reovirus



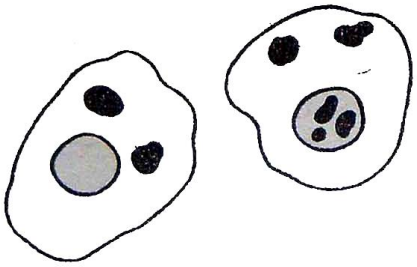
Cells infected by rabies virus (Negry bodies)



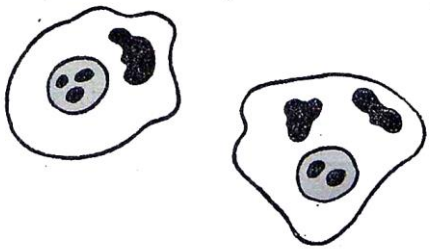
Cells, infected with adenovirus



Cells, infected with papovavirus SV-40

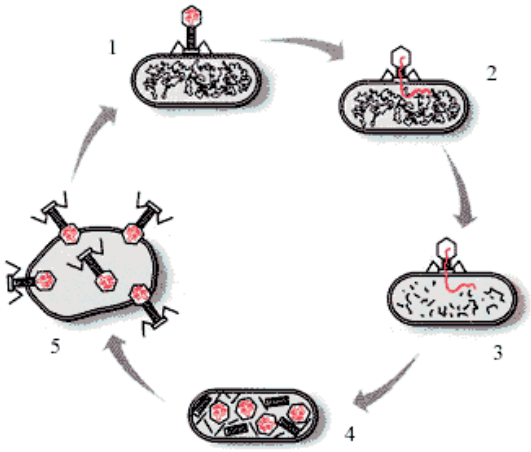


Cells, infected with influenzavirus



Cells, infected with morbilivirus

Fig. 14. Intracellular inclusions in viral infections



1._____

2._____

3._____

4._____

5._____

Fig. 15. Interaction of bacteriophage with sensitive bacterial cell.

Virology Laboratory is used for laboratory diagnostic tests for viral infections, monitoring and intensity evaluation of specific post-infection and post-vaccination immunity, and participates in the prevention of viral diseases. The structure of the virology laboratory depends on the objectives and features of its activities. Typically, virology laboratories, regardless of specialization, must be placed in a clean bright premises with the necessary equipment and furniture. They should be separated from other laboratories (bacteriological, toxicological, etc). Besides everyday routine cleaning, facilities periodically should be treated with the disinfectant solution (carbolic acid, bleach, etc.) and subjected to ultraviolet irradiation. Rooms designed for work with viruses, should be well lit and composed of two compartments, separated with glass: box and prebox. In the box, organized similar to surgery room the working table made of stainless steel should be placed and at a distance of 50-70 cm from its surface ultraviolet germicidal lamp should be installed. At the entrance of the box special mat impregnated with disinfectant solution must lie. In prebox in the special container the disposable slippers, sterile gowns, caps, masks, gloves are placed. Boxes and preboxes except daily wet disinfection are usually irradiated by bactericidal lamps for 30-40 minutes before and after work. In the box (prebox) there must be all necessary equipment: thermostats, refrigerators, laboratory centrifuges, microscopes, water bath, sterilizer for tools, cans with disinfectant solution for used pipettes, a tank with a disinfecting solution for the collection of waste materials, cans of alcohol, iodine, test tube racks, test tubes with broth for testing bacterial contamination, sterile pipettes, etc. The ceiling and walls of the laboratory rooms should be covered with an oil paint. The laboratory should have 5-6 rooms, separate entrance for employees and visitors with a vestibule, cloakroom and shower required. Registration of pathological materials for diagnostics is carried out in the waiting room. Floor in the premise should be of concrete, the walls be covered with oil paint or ceramic tiles, windows sealed with iron bars. It should be (if of need) a special box with prebox connected to a vivarium for infected animals handling.

In box newly arrived materials are evaluated, fixed smears and print-smears are prepared. The room should also has tables for necropsy and processing of the material with all necessary instruments (scissors, forceps, scalpels,

etc), enameled basin, filled with disinfectant, sterile container to collect the material. Room for virological research should be installed with different tools: thermostats, refrigerators, centrifuges, scales of various types of hot plates, filters, pumps, water baths, electromagnetic stirrers, etc. and laboratory supply.

Vivarium is called the premises used for the breeding of laboratory animals. In the vivarium they usually organize

- quarantine section,
- rooms for experimental and healthy animals,
- cleaning premise for disinfection of cages, equipment and clothing,
- kitchen for Preparation of food,
- crematorium for dead animals destroing, etc.

All rooms of vivarium should be isolated from each other and have independent enters. Furthermore, they must be impermeable for various rodent and insects. The vivarium should be warm, light (with a source of daylight) and dry. In the cold season the temperature should be about 12-20 ° C. Laboratory animals are kept in cages. In one cage there maybe 1-2 rabbits, 5-6 guinea pigs, 8-10 rats, 15-20 mice. On each cage the label should be fixed with information about the experiment: date, number of animals, mass, nature of infection, family name of the doctor.

Cleaning of the cages and vivarium premises should be done every day, using soap and carbolic solution. After cleaning the staff should thoroughly wash hands and treat them with a 2% solution of bleach.

Employees of the virology laboratory must provide the following rules that prevent contamination of the test material with bacteria and fungi from laboratory staff and possible spread of infection.

1. Permitted to work in special clothes only.
2. Forbidden to go outside the lab in special clothes or wear something over it.
3. No smoking or eating in the laboratory.
4. Not allowed walking, talking while working.
5. All material is fed to the laboratory for analysis, should be considered as positive.
6. You should be careful when unpack the material. Cans should be wiped outside with disinfectant be placed on special tray.
7. The transfusion of fluids that contain viruses, produce over a vessel filled with disinfectant solution.
8. In case of contact with infectious material on the robe, hands, table, shoes, etc. immediately inform the Head of Laboratory (in education - teacher) and treat contaminated area with appropriate disinfectant /antiseptic under his control. Consult your doctor if need.
9. Contaminated material is subject to mandatory destruction (autoclaving), if possible on the same day. Tools, as well as the desktop after work are immediately disinfected.
10. The logbook entries are made daily about using and destruction of infectious materials.
11. The imposition of lab equipment, tools, materials, etc. from the laboratory must be prohibited without preliminary disinfection in place.
12. Pipettes, slides, cover glass and other things should be disinfected by immersion in a 5% solution of Lysol, phenol or sulfuric acid, which must always be on the table.
13. Do not leave on the desktops bacteriological dishes, tubes and other utensils with infectious materials.
14. At the end of the work the working area should be cleaned and thoroughly disinfected. Virus-containing material, necessary for further work, is put into storage (the safe-refrigerator) and seal. Hands should be thoroughly disinfected with soap and 70 °alcohol. For all virological laboratories a uniform procedure should be established for the treatment of viruses, providing rules for their storage, registration, transfer from one employee to another within the lab and beyond. Virus culture is stored in the refrigerator below (-20 ... -70 ° C) locked and sealed. All the tubes and vials, closed with rubber stoppers, should have labels, indicating type of viruses, strain and stabilizing environment. The best way to keep viruses is lyophilization.

Virology laboratory should have the following **documentation**:

1. Museum Inventory book of virus strains.
2. Journal for registration of motion of virus-containing material in the laboratory.
3. Journal for registration of sterilization and destruction of infected material.
4. Journal for registration of infected laboratory animals.
5. Journal for registration of virology examinations.
6. Journal for registration of viruses isolated.

Virological investigation^ general principlesw: means isolation of the virus from pathological material, its serological identification and detailed study of various properties (pathogenicity, antigenicity, cultivation in the laboratory, morphological features). In every case of the disease with a suspected viral etiology it is necessary to isolate the agent from pathological material first. In this regard, the correct selection, packaging, transportation and processing of the material is of great importance for the successful diagnosics of the viral disease.

The material for the study. From diseased, dead or slaughtered animals the material should be taken as soon as possible after the onset of clear signs of illness or no later than 2 ... 3 hours after clinical death or slaughter. The material should be taken with regard to suspected disease pathogeneses (the entrance gate, the spread in the body, the place of reproduction and the ways of excretion). For example, in respiratory infections for virus isolation they take nasopharyngeal swabs, nasal swabs and throat swabs of the trachea and lung slices of corpses, in enterovirus infections - cal, in neurotropic infections - pieces of the brain or spinal cord, etc., ie, select the material claimed to contain the heist virus concentration. The material for virus isolation may serve a variety of excreta and secrets, pieces of organs,

blood, lymph, etc. At autopsy of animals material is collected under strict aseptic and antiseptic rules in order not to contaminate the material and do not allow the infection to spread. Blood is taken from the jugular vein, the tip of the tail or ear, venous plexus of the eye, etc. For virus isolation whole defibrinated, "lacquer" blood (blood mixture with distilled water in the ratio 1:1) or individual elements of blood (red cells, white cells, plasma, serum) can be used. For the detection of antiviral antibodies blood is taken from the same animal twice at intervals of 2 ... 3 weeks (for paired sera of not less than 5.0 ml each).

Washings from the mucous membrane of the nasal cavity, eyes, pharynx, rectum and cloaca (in birds) are taken by sterile cotton swab and immersed them in the vials or tubes containing 3.5 ml of transport solution. For this purpose commonly Hank's solution or tissue culture medium "199" with antibiotics (penicillin 500 IU, streptomycin 500 IU and nystatin 20 IU per 1 ml of medium) with a protein stabilizer such as 0.5% gelatin solution or 0.5 ... 1% solution of bovine serum albumin. The presence of stabilizers prevents rapid inactivation of certain viruses, i.e. parainfluenza virus.

Faeces are taken from the rectum with a spatula or a stick and placed in a sterile vial. Vesicular fluid is collected with a syringe in a sterile tube. Spinal fluid taken aseptically by conventional puncture. As the pathological material used pieces of tissues (few cm3 and weighing 10-20 g), which are:

a) shows visible abnormalities in shape, size, color, consistency, presence of unusual formations;

b) may be affected and contain the virus;

c) most often contain a virus - the liver, spleen, lungs, brain, lymph nodes, kidneys.

Transport and storage of samples.

Samples taken should be put as quickly as possible in conditions that ensure the retardation of the virus inactivation. Such conditions mean low temperature. For this tubes with material, closed with rubber stoppers are placed in cooling mixture.

The structure of the packaging container:

Initial capacity: container containing the sample (sample vial with a screw cap and non-toxic rubber seal, wrapped with adhesive tape or sealed ampoules);

Inner packing: - absorbing material - tissue paper or cotton wool in a quantity sufficient to absorb the liquid in the event of a leak; a plastic bag sealed or glued with adhesive tape;

Outer packing: anti-shock-pad (crumpled paper or wadding);

- solid water-tight container with tight-fitting lid.

As the cooling mixture an equal parts of dry ice (solid carbon dioxide) and ethanol are used. It allows to keep the temperature of minus 71 ° C for several days. You can use a mixture of three parts (by weight) of ice or snow, and one part of salt. In the latter case it is possible to obtain the temperature of minus 15 ... 20 °C. Instead of freezing, you can use chemical preservatives, but it is less effective. The best of them is a mixture of equal volumes of sterile glycerol and 0.85% sodium chloride (normal saline). Typically, the mixture is recommended to use for preserving pieces of parenchymal organs and tissues. Using glycerol based solutions makes it impossible to study the pathological material by immunofluorescence. In this case an independent sample of pathological material should be prepared by methods suitable for fluorescent microscopy. Pathological material should be provided with reliable and accurate label (Fig. 1). They should indicate personal data, type of material, date and destination. Accompanying document should contain full details of the patient from whom the samples were taken, of epidemiologic data, a presumptive diagnosis, as well as the data concerning doctor and medical institution. These data are very important when choosing the direction of laboratory research. Material delivered in a laboratory should be immediately used for virus isolation. If for some reason (lack of experimental animals, chick embryos, cell cultures) investigation is delayed, the material is stored at minus (40 ... 70) °C. Most viruses in the blood, cerebrospinal fluid, urine, nasal swabs and scrapings are quickly destroyed, so the success of their isolation depends on the speed of the investigation. If there is no confidence that the diseases is caused by the virus alone, the material should be given to bacteriological or/and mycological research.

Preparation of virus-containing material.

In the laboratory the pathological material obtained should be freed of preservatives, thawed and washed from glycerol, weighed or measured. Part of the material is designated for virological analysis, the remainder - stored in refrigerator in case you need additional study. Then a plan of investigation of the material is created. Materials for infection of contamination-sensitive objects is carried out in two ways: with the antibiotics treatment or by sterile filtration.

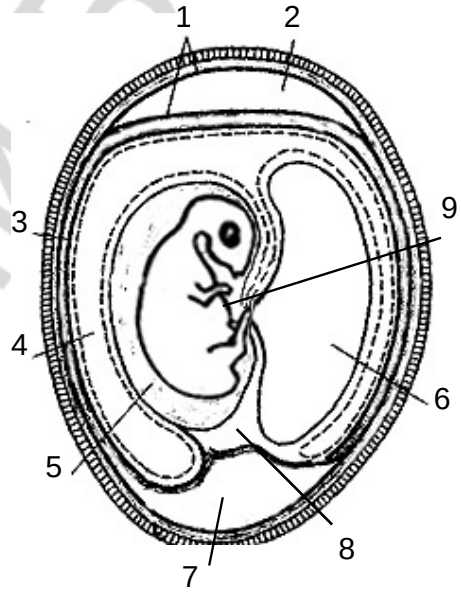
Preparation of organs and tissues. The virus should be freed from the cells of organs and tissues to Hank's solution. To do this, the material is thoroughly cut with scissors and grated in a mortar with sterile quartz sand. From the ground material is usually prepared 10% suspension in Hanks solution. The resulting suspension is centrifuged at 1500 ... 3000 rpm for 15 ... 30 minutes, the supernatant is transferred into sterile vials and freed from bacteria by broad-spectrum antibiotics (Penicillin, Streptomycin, Nystatin, Tetracycline, etc.) treatment. Doses of antibiotics used for this purpose can vary within a wide range (from 100 to 1 ... 2 million IU or more per 1 ml) depending on the nature of the material used. Exposure to antibiotics should be no less than 30 ... 60 min at room temperature, then the material is subjected to a bacteriological control. After a negative result of bacteriological control the virus-containing material can be used to infect laboratory animals, chick embryos and cell cultures. In case of a positive bacteriological control virus suspension is subjected to further processing. The suspension was stored at minus (20 ... 70) °C.

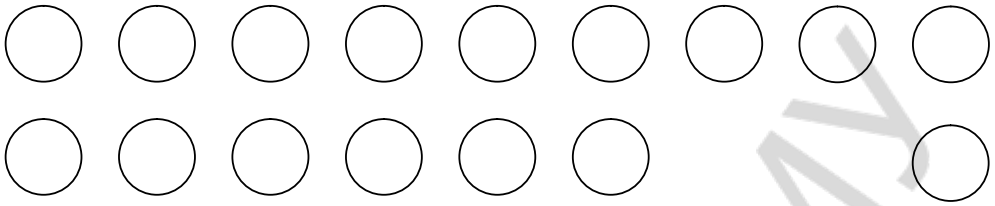
Class № 2. **Date_____**

Topic: Virologic diagnostics of diseases caused by ortho- and paramyxoviruses

List of questions to study:	Medical Microbiology / F. H. Kayser, K. A. Bienz, J. Eckert , R. M. Zinkernagel // Thieme New York 333, Seventh Avenue, New York, NY 10001 USA.- 2005.:
1. Orthomyxoviruses. Taxonomy and characteristics of the family. Influenza viruses A, B, C. Morphology. Antigenic structure and serotypes. Antigenic diversity (shift and drift) and its consequences. Influenza, prevalence, pathogenesis, immunity. Methods for influenza diagnostics. Principles of therapy and prophylaxis, preparations for specific immunoprophylaxis and chemopherapy of influenza. Avian and swine influenza. 2. Paramyxoviruses. Taxonomy and characteristics of the family. Differentiation with orthomyxoviruses. Parainfluenzaviruses, properties, importance for human pathology. Pathogenesis, immunity, diagnostics. Mumps virus, properties, pathogenesis, immunity, specific prophylaxis. Morbilivirus, morphology, properties, pathogenesis, immunity, specific prophylaxis. HRSV, properties, importance for human pathology..	1. P. 458 – 460; 2. P. 464 – 467.

Laboratory work

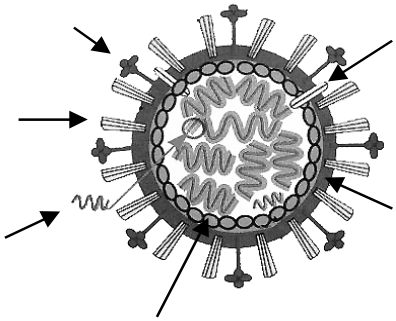
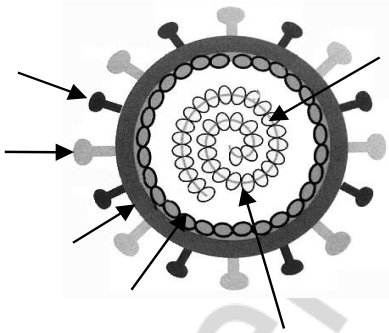
Exercises	**Laboratory report**	
1. Chicken embryo inoculation with influenzavirus in allantois cavity.	1.Study the structure of hen embryo (8-11 days) 2. Examine hen embryo in ovoscope and determine the vitality signs: а) the dimensions of the embryo shape б) presence of the developed blood vessels pattern в) active mobility of the embryo г) mark the air cavity border 3. Set embryo on the egg rack and treat shell as follow: а) 70% alcohol б) 5% iodine 4. Inoculate embryo as follow: а) flame scissors б) carefully pierce the shell 3-5 mm above an air cavity border в) take 0,2 ml of viral material (live influenza vaccine) in the syringe г) put the needle into the embryo (25 mm) vertically and introduce the material. 5. Repeat shell treatment according to p.3. 6. Seal the shell with tape or melted wax. Mark the embryo (group number). Inoculation of the Allantoic cavity : 1. Use cotton wool and 70 percent alcohol to swab the end of the eggs to be inoculated. Allow the alcohol to evaporate. 2. Swab the eggshell punch with 70 percent alcohol solution. Place used cotton wool in discard tray. 3. Pierce a hole in the end of the egg at the marked inoculation site. 4. Attach needle to 1 mL syringe. 5. Draw inoculum into 1 mL syringe. 6. Keeping the needle and syringe vertical, place the needle through the hole in the eggshell approximately 16 mm into the egg to reach the allantoic cavity. 7. Inject 0.1 mL of inoculum into the egg. 8. Withdraw the needle from the egg. 9. Seal the hole in the shell with stationery tape or melted wax. 10. Discard the used needles and syringes. 11. Place the inoculated eggs into an incubator.	 1. Shell membrane 2. Air sac 3. Chorioallantoic membrane 4. Allantois cavity 5. Amnion cavity 6. Yolk sac 7. Albumin 8. Extraembryonic cavity 9. Embryo

Demonstration 1. Preparations for specific prophylaxis and therapy for influenza and measles 2. HIT with pared sera for influenza serodiagnostics.	**HIT in paired sera** 1/2 1/4 1/8 1/16 1/32 1/64 EC VC SC 

Tutor signature_____

Additional materials to class № 2.

Write in virus family name, indicate respective structural elements of virion

The structure of _____virus.  1. Haemagglutinine 2. Neuraminidaze 3. Supercapside 4. Matrix protein M1 5. Protein M2 6. RNA	**Influenza diagnostics by virus isolation in chicken embryo *** 1. Clinical material are taken during first three days of the disease with sterile cotton swabs. Swabs are washed in saline and disposed appropriately. Viruses can be concentrated by adsorbtion to guineas pig erythrocytes. Any material should be treated with antibiotics (e.g. penicilline +streptomycine) for one hour and tested for sterility before cell culture or chicken egg inoculation. 2. Embryo inoculation 3. Incubation for 3-4 days at 35˚C 4. Sampling of embryo liquids and tissues (see next class). 5. Virus reproduction indication by HT. 6. Virus identification in HIT, NTИ or other tests.
The structure of _____virus.  1. Glicoprotein F 2. Glicoprotein HN, G 3. Supercapsid 4. Matrix protein 5. Nucleocapsid 6. RNA	**Serological influenza diagnostics** Serological diagnostics of influenza is usually performed in paired sera by CFT or HIT. CFT can reveal serotype-specific antibodies while HIT allows to discriminate between strains in a particular serotype. The set of typical influenza strains should be used as an antigen. 1. Sera preparing. 2. Virus standard titration (determination of agglutination activity) 3. HIT. 4. Titer determination and conclusion.

Hemagglutination inhibition test for antibodies detection against influenzavirus type A

	Reagents	Well number								0,1 Controls		
		1	2	3	4	5	6	7	8	Serum	Erythrocytes	Virus
utions		**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**			
ng		0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,2	0,1
ent`s serum		0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1		
us 4HU		0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1			0,1
	Incubation for 20 min at room temperature											
	1% chicken erythrocytes	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
	Incubation for 60 min at room temperature											
ults		-	-	-	-	-	+	++	+++	-	-	+++

Result interpretation

When specific antibodies are present they neutralize viral haemagglutination activity. In positive wells erythrocytes settle down and form compact sediment ("button"). In negative wells typical agglutination is observed. HIT allow to reveal specific antibodies and to determine its titer. The titer in HIT is the lest serum dilution wich can completely block haemagglutination. This example gives the titer 1/32.

Laboratory diagnostics of mumps

1. Usually is not needed because the symptoms are very characteristic.
2. Used for diagnostics when:
   - The disease is atypical with damage of internal organs and glands (pancreatitis, thyreoiditis, orhytis)
   - There is the need to discriminate between other reasons of saliva glands damage.
3. Serologically antibodies titer increment in paired sera by ELISA (CFT, HIT)can be determined.
4. Virus isolation: virus can be isolated from saliva (first 3 days of the disease), liquor (6 days) and urine (9 days):

а) mumps virus grows in chicken embryos (7-8 days old). Inoculation is performed in amnion cavity. Embryos then are incubated for 6-7 days at 35° C. For virus indication HA is used;

б) virus olso can be isolated on cell culture (human embryo kidney cells, HELA). Indication is performed by CPE registeration after 48-72 hours of incubation (giant multinucleated cells and symplasts with cytoplasmic inclusions). Later cellular monolayer is fully destroyed;

в) for identification of isolated viruses – FAT, HIT, NA or CFT are used.

5. Molecular-genetic methods (PCR).

Laboratory diagnostics of measles

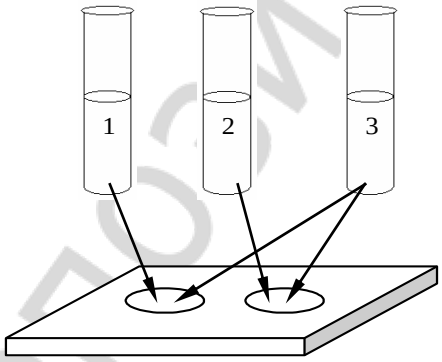
1. Usually is not needed because the symptoms are very characteristic.
2. Used for diagnostics when:
   - The disease is atypical;
   - epidemic outbreak investigation;
   - lethal cases investigation.
3. Examination of nasopharyngeal smears by FAT, detection of characteristic multinuclear cells. Rash elements scraping can also provide material for smears.
4. Virus can be isolated from blood or nasopharyngeal wash from prodromal stage till first day after rash appearance. Human embryo kidney cells, Vero or other cells are inoculated. After 3-4 days of incubation characteristic CPE can be registered: giant vacuolated multinucleated cells and cyncytium with cytoplasmic inclusions. Also round-cell or spindle-cell degeneration with cytop;asmic or nuclear inclusions can be observed. Identification of isolated viruses is performed by FAT, HIT or NA.
5. Serologically antibodies titer increment in paired sera by ELISA (CFT, HIT) can be determined.
6. Molecular-genetic methods (PCR).

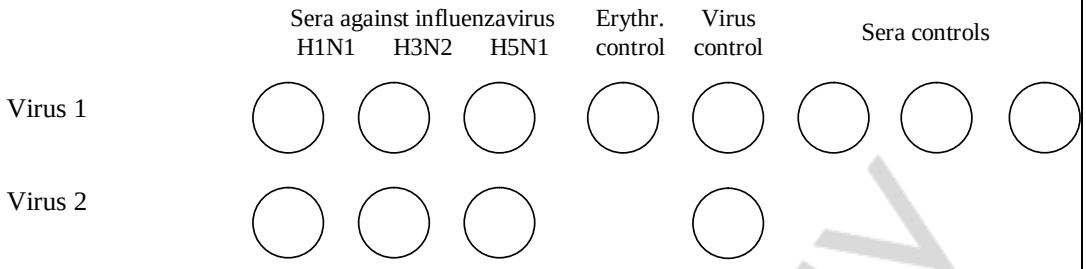
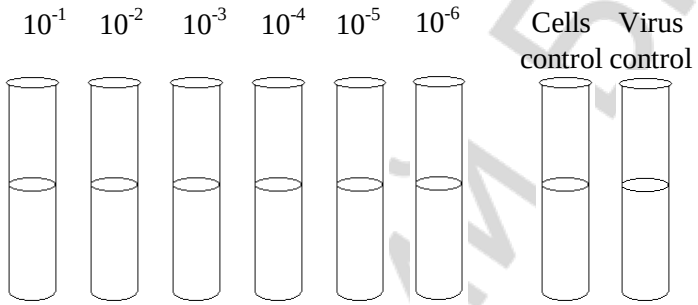
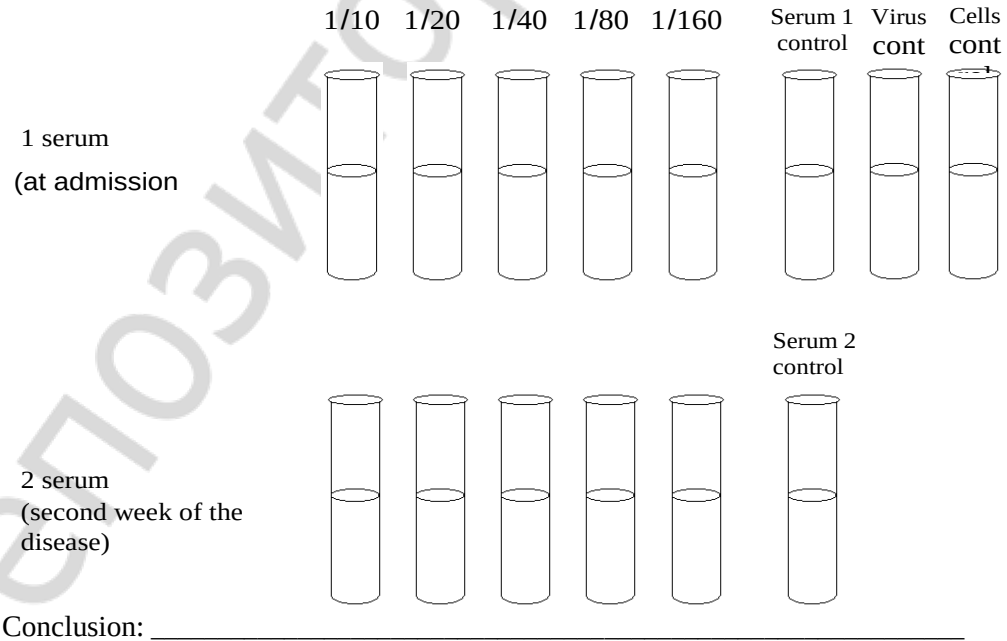
Class № 3. **Date_____**

Topic: Methods of diagnostics for diseases caused by picornaviruses, rotaviruses and retroviruses.

List of questions to study:	Medical Microbiology /
1. Picornaviruses. Taxonomy and characteristics of the family, importance for human pathology. Etiology, pathogenesis, immunity, diagnostics and immunoprophylaxis of poliomyelitis. Problem of poliomyelitis eradication. Coxsackieviruses and ECHOviruses, importance for human pathology. Methods for discrimination. Rhinoviruses. Taxonomy. Structure and characteristics. Prevalence, pathogenesis, immunity. 2. Rotaviruses, characteristics, role in pathology. 3. Retroviruses. Taxonomy and characteristics of the family. Human immunodeficiency virus (HIV-1, HIV-2). Morphology. Pathogenesis, role of CD4+ and CD8+ T-cells. AIDS-associated diseases. HIV diagnostics, prophylaxis, treatment. HIV in Belarus.	F. H. Kayser, K. A. Bienz, J. Eckert , R. M. Zinkernagel // Thieme New York 333, Seventh Avenue, New York, NY 10001 USA.- 2005.: 1. P. 434 – 437 2. P.455 – 457 3. P. 448 - 455

Laboratory work

Laboratory exercises	**Laboratory report**
1. Chicken embryo autopsy.	1. Before autopsy embryo should be cooled for 2-3 hours at 4–6° C for blood vessels constriction. 2. Treat the egg shell with 70%-alcohole and flamed. Repeat it once more. 3. Open the shell by sterile scissors 2-3 mm above air sack border. Remove shell membrane and aspirate 1 ml of allantois cavity liquid. 4. Amnion cavity liquid can also be taken (0,5-1,5 ml). 5. Remove an embryo on the Petri plate. Allantois membrane should be carefully examined by yes. Usually influenzaviruses produce no CPE. 6. Perform slide HT for virus indication **Slide HT** Put two drops of 5% chicken erythrocytes suspension onto glass slide. Add and mix one drop of allantois liquid (experiment) and saline (negative control) with each drop. The test is positive if flakes of erythrocytes are developed. The test is negative if erythrocytes remain in suspension after 5-7 min.
2. Virus indication by HT	**Slide HT scheme**  1. Allantois liquid 2. Saline 3. 5% chicken erythrocytes Conclusion:_____

3. Evaluation of HIT for influenzavirus identification.	**Evaluation of HIT results for influenzavirus identification**  Conclusion:_____
Demonstration 1. Poliomyelitis virus titration by color test.	**Determination of poliomyelitis virus titer by color test**  Conclusion_____
2. Neutralization test on cell culture in paired sera for poliomyelitis serodiagnostics.	**NT in paired sera for poliomyelitis serodiagnostics**  Conclusion: _____ _____

Tutor signature_____

Additional materials to class № 3.

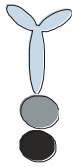
ELISA for HIV infection screening

At present the fourth generation ELISA kits are used. Its main advantages include recombinant antigens, monoclonal antibodies, simultaneous detection HIV antigens (usually p24) and antibodies against surface HIV antigens

Biotin and avidin represent a pair receptor-ligand with very high affinity and specificity. Their properties allow to use this for antigens and antibodies tagging. One avidin molecule can bind four biotin molecules.That is the signal about binding would be four times higher.

The scheme of ELISA for HIV infection screening

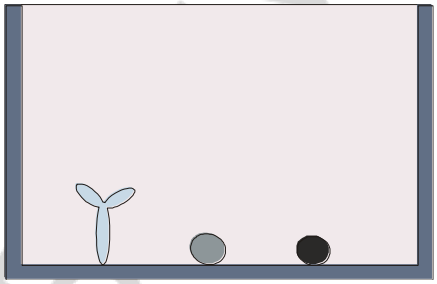
1. In wells of the plate for immunoanalysis certain ingredients are adsorbed:



Monoclonal antibodies to p24

Recombinant epitop from gp41,

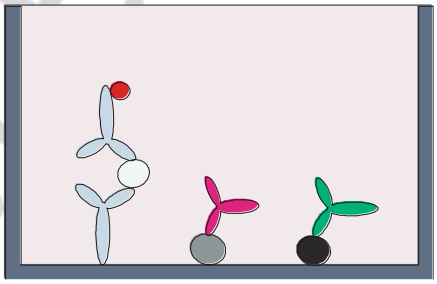
Recombinant epitop from gp120



2. When patients serum is added p24 and antibodies against surface HIV glicoproteins bind their adsorbed ligands.

p24

Antibodies to HIV glicoproteins from patients serum



3. Then conjugates are added:

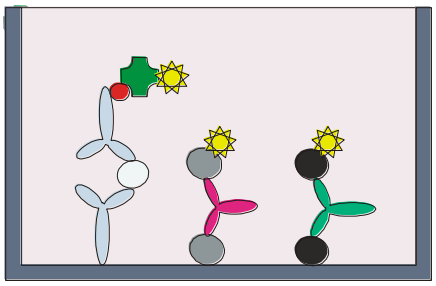
Antibodies to p24 +biotin

gp41+peroxidase

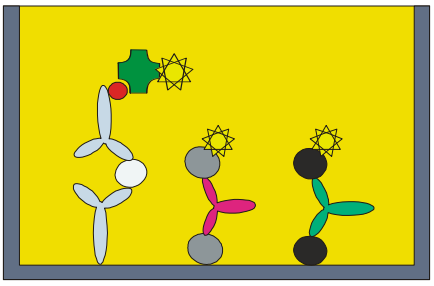
gp120+peroxidase

Avidin+peroxidase

Conjugate molecules fix to immune complexes according to the quantity of molecules to reveal.

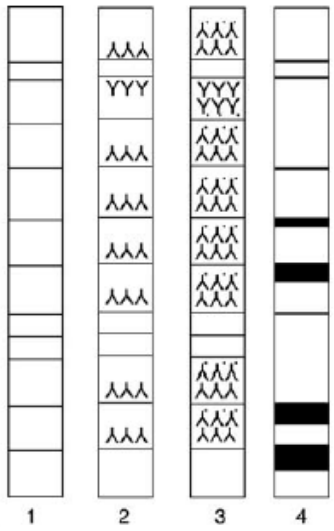


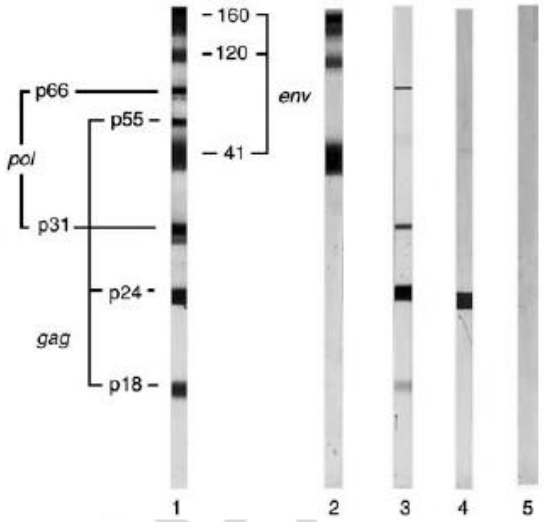
4. After substrate is added the dose-dependent fermentation occurs and colored product is developed.



Color test. was proposed by Salk, Youngner, and Ward for screening large quantities of materials for poliomyelitis viruses. The test is based on the phenomenon of virus induced inhibition of cell culture metabolic activity. Healthy, uninfected culture gradually reduces the pH of the medium, and if phenol red has been added as an indicator, the color changes from red to yellow. If the kidney cells are killed by poliomyelitis virus, metabolism ceases and the medium remains red. Conversely, if antibody-containing serum is added to virus and susceptible cells, the virus is neutralized and does not kill those cells with the result that metabolism proceeds in a normal manner and the medium becomes yellow. Titrations of either serum or virus can thus be readily carried out and satisfactory readings are usually possible by the seventh day. Usually color test is applicable only for highly cytopathogenic viruses like enteroviruses or adenoviruses.

Immunoblotting for HIV infection diagnostics



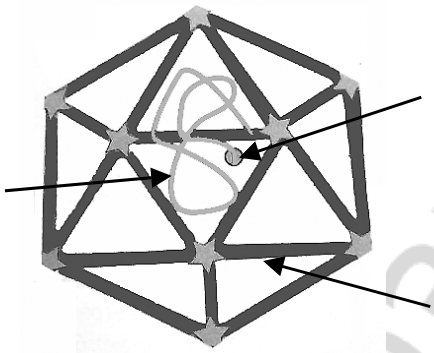


1. Blot preparing: electrophoretic separation of HIV proteins by their mass and charge. Transfer to the membrane, fixation and cutting into strips.
2. Incubation with serum examined. Washing.
3. Incubation with antibodies, tagged with peroxidase, against human antibodies
4. After substrate is added color bands appear where patient`s antibodies bind to HIV antigens.

1. Positive result in person, infected with HIV-1
2. Healthy person, vaccinated with surface HIV-1 glicoproteins
3. Uncertain result in person, infected with HIV-2
4. Uncertain result because of some unrelated antibodies presence cross-reacting with p24 antigen
5. Negative result

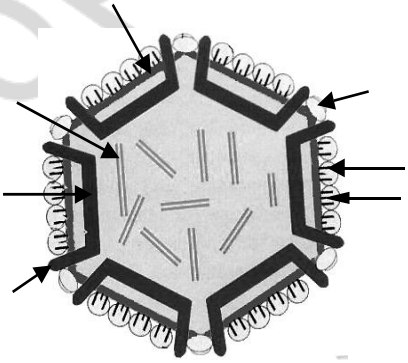
Write in virus family name, indicate respective structural elements of virion

The structure of _____virus.



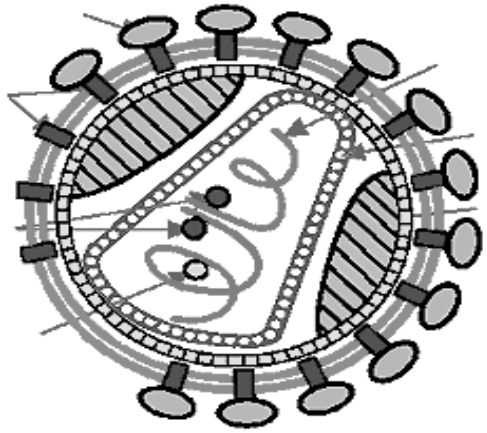
1. Capsid
2. VPg
3. RNA

The structure of _____virus.



1. External capsid
2. internal capsid
3. VP4
4. VP6
5. VP7
6. RNA
7. Gp M-1C

The structure of _____virus.



1. Capsid (p24)
2. Nucleocapsid (p6, 9)
3. Matrix protein (p17)
4. Revertase (p55, 63)
5. Integrase (p11)
6. gp120
7. gp41

Class № 4. **Date_____**

Topic: Virological diagnostics of diseases caused by arboviruses and roboviruses. Oncogenic viruses. Slow infection.

List of questions to study: general features of arboviruses. 1. Toga-, flavi-, bunja-, arenaviruses, naxonomy, virion structure, role in human pathology. Etiology, pathogenesis, immunity, methods for tick encephalitis diagnostics. Hemorrhagic fever with kidney insufficiency syndrome (HFKS or HFRS). 2. Rubella virus. General characteristics. Role in human pathology. Prophylaxis. 3. Rabdoviruses. Taxonomy and characteristics of rabdoviruses. Pathogenesis, immunity and specific prophylaxis of rabies. 4. Filoviruses. Marburg and Ebola viruses. 5. Oncogenic viruses (DNA and RNA). Viral cancerogenesis mechanisms. 6. Slow infections etiology.	Medical Microbiology / F. H. Kayser, K. A. Bienz, J. Eckert , R. M. Zinkernagel // Thieme New York 333, Seventh Avenue, New York, NY 10001 USA.- 2005.: 1. P.440 – 445; 460 - 464; 2. P. 440; 3. P. 467 – 470; 4. P. 471; 5. P.413 – 417; 424 – 426; 448; 6. 472.

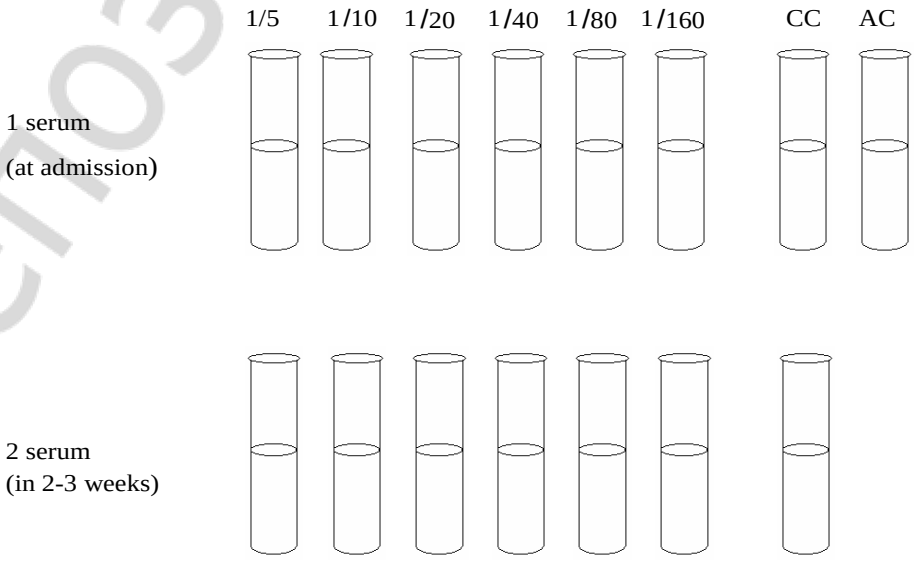
Laboratory work

Laboratory exercises	Laboratory report
1. Determination of titer increment in paired sera for tick encephalitis diagnostics	CFT performance scheme

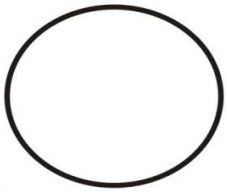
Reagents	1	2	3	4	5	6	7	8	9
	1/5	1/10	1/20	1/40	1/80	1/160	AC	CC	HS
Saline	-	0,5	0,5	0,5	0,5	0,5	0,5	0,5	5 ml 3% erythrocytes suspension + 5 ml hemolytic serum
Patient sera*	0,5	0,5					-	0,5	
Diagnosticum	0,5	0,5	0,5	0,5	0,5	0,5	0,5	-	
Complement	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	
Incubation 30 min at 37°C									
Hemolytic system	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	
Incubation 30 min at 37°C									
Result evaluation									

*Test is performed in two rows with first and second patient`s sera respectively.

CFT results evaluation for tick encephalitis diagnostics



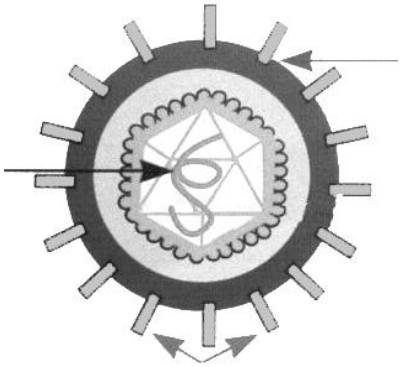
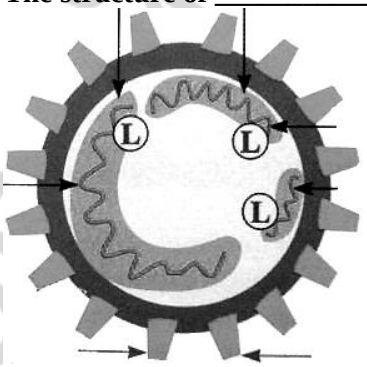
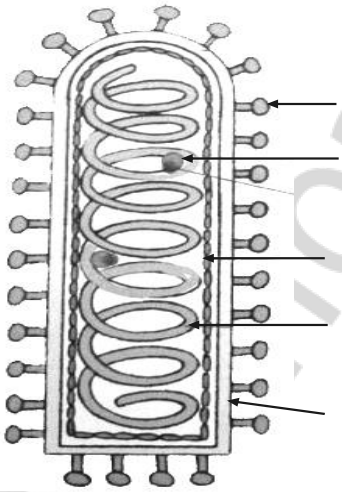
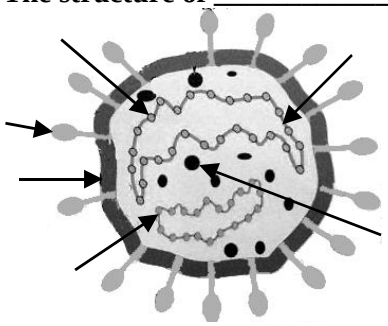
Conclusion: _____

Demonstration 1. Negry bodies in mouse brain homogenate (Muromtcev stain).	 **Smear** _____ _____ **Stain**_____

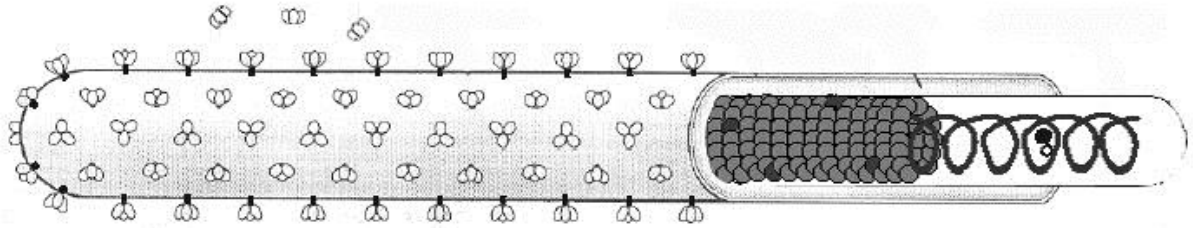
Tutor signature_____

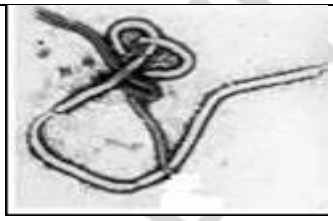
Additional materials for class № 4.

Write down family name and mark virion elements with suitable number.

The structure of _____virus.  1. Supercapsid 2. Capsid 3. RNA 4. Glycoproteins	**The structure of _____virus**.  1. Nucleocapsid 2. Supercapsid 3. Glycoproteins 4. M-RNA 5. S-RNA 6. L-RNA
The structure of _____virus.  1. Supercapsid 2. Nucleocapsid 3. Glycoproteins 4. Polymerase 5.Matrix protein	**The structure of _____virus**.  1. Supercapsid 2. Nucleocapsid (beads on RNA) 3. Ribosomes 4. L-RNA 5. S-RNA 6. Glicoproteins

The structure of _____virus.



1. Glycoprotein (gp1+gp2) 2. Soluble glycoprotein 3. Supercapsid 4. M-protein (p40+p24)	5. Nucleocapsid 6. Nucleoprotein (NP) 7. Polymerase (p30+p35) 8. RNA	

Tick encephalitis diagnostics

1. Materials for investigation: blood, urine, liquor, brain (autopsy). For serological diagnostics paired sera are used (first is taken at the beginning and second - on 5^{th}-7^{th} week of the disease.
2. Virus can be isolated on white mice: prepared material is injected in brain directly. After 8-12 days symptoms can be registered (irritability, unsteadiness, convulsions, paralysis, death). If no disease appear two more passages can be done.
3. Virus can be isolated on cell culture (chicken embryo fibroblasts and others). TEV usually do not produce CPE.
4. Indication/identification is performed by FAT, NT on mice, HIT or CFT with standard typospecific sera.
5. Serological tests include CFT, PHAT, HIT and most often ELISA.

Rabies diagnostics

1. Materials for investigation: brain, salivary gland tissues, skin biopsy, hair follicles. 2. Diagnostics is based on the detection of Negry bodies or viral antigens in tissues sections or smears. Inoculation of white mice is also used:
a) Negri bodies are eosinophilic, sharply outlined, pathognomonic inclusion bodies (2–10 μm in diameter) found in the cytoplasm of certain nerve cells containing the virus of rabies, especially in Ammon's horn of the hippocampus. Often also found in the cerebellar cortex of postmortem brain samples of rabies victims. They consist of ribonuclear proteins produced by the virus. The sections are usually stained by Mann's, Giemsa, or Sellers methods which permit differentiation of rabies inclusions from other intracellular inclusions. With these stains, Negri bodies appear magenta in color and have small (0.2 μm to 0.5 μm), dark-blue interior basophilic granules. In former SU countries brain sections are stained by Muromtcev method for rabies diagnostics
б) FAT allow to reveal viral antigens in cytoplasm of the infected neurons (in brain, salivary glands, skin, hair follicles etc). When fluorescein is used for antiserum targeting viral antigens appear as greenish granules of different size (0,2 - 25 mkm).

Oncogenic viruses

Oncogenic viruses are able to transform (make cell immortal and cause genome instability and tumor progression) normal cells in vitro and in vivo.

Transformation signs:

- Adhesion loss
- increasing mobility
- invasive activity
- resistance to proliferation and differentiation control mechanisms
- ability to form tumors
- increased rate of chromosome aberration

RNA oncogenic viruses

Oncogenic RNA viruses belong to 5 genera of *Retroviridae* family, *Oncornavirinae* subfamily — *Alpharetrovirus* avium myeloblastosis virus (AMV), Rous sarcoma virus — RSV), *Betaretrovirus* (Mouse mammary tumor virus— MMTV), *Gammaretrovirus* (Murine leukemia virus — MuLV), *Deltaretrovirus* (Human T-lymphotrophic virus, Bovine leukemia virus — BLV, HTLV), *Epsilonretrovirus* (Walleye dermal sarcoma virus — WDSV).

Mechanisms of oncogenic transformation include introduction in a cell of highly active oncogenes (normal cell genes homolog which are able to transform cells in culture. Viral oncogenes are designated v-onc, and respective cellular oncogenes - c-onc. At present many oncogenes are identified and their functions studied (growth factors and their receptors, G-proteins, signal factors, transcription factors, regulators of apoptosis and cell cycle etc.).

Some RNA oncogenic viruses can transform cells without oncogenes by specific integration:

- by enforcement of normal cellular genes activity (IL2, IL2R, c-fos) with viral promotor;
- by damaging of antitumor genes activity (RBp, p53 etc.).

DNA oncogenic viruses

DNA oncogenic viruses belong to 5 families: polyomaviruses, papillomaviruses, adenoviruses, herpesviruses, hepadnaviruses. Of course not all viruses from particular family are oncogenic and not all oncogenic viruses can induce advanced tumors (see table below).

DNA oncogenic viruses use similar transformation mechanisms:

- increase of cellular genes activity (translocation or specific integration). Cellular oncogenes are activated by strong viral or cellular promoters (e.g. TCR or immunoglobulines).

Tumor	C-onc	Promotor
Burkitt lymphoma	Myc	Heavy and light chains of immunoglobulines
Chronic B-cell lympholeucosis	Bcl1, bcl2	Heavy chain of immunoglobulines
Chronic T-cell lympholeucosis	tcl1	TCR
Chronic T-cell lympholeucosis	Myc	TCR

- introduction of highly active oncogenes in the cell:

DNA oncogenic viruses have their own oncogenes

Virus	Oncogen(propein)
Adenoviruses	Region E1A
SV40	Major T-ag
Polyomavirus	Major T-ag
Lymphocytotropic viruses	Major T-ag
Human papillomavirus 16	E7

The transformation mechanism of DNA oncogenic viruses includes violation of cellular apoptosis and leads to cell immortalization and tumor progression. Many viruses express mechanisms damaging the function of antitumor cellular factors: adenoviruses bind and neutralized retinoblastoma gene protein; HCV binds antioncogene p53, and papillomaviruses can target it and destroy on proteosomes.

DNA oncogenic viruses

Family, virus	Ability to transform cell culture	Ability to induce tumor in animals	Ability to induce cancer
Polyomaviruses:			
Pv mouse	+	+	-
SV40	+	+	-
HBKV	+	+	-
HJCV	+	+	-
Papillomaviruses:			
animal	+	+	-
avium	+	+	-
human	+	+	Cervical cancer
Adenoviruses:			
animal	+ (all)	+ (C-E)	+
human	+	+	-
Herpesviruses:			
HSV	+	-	-
CMV	+	-	-
EBV	+	+	Burkitt lymphoma, nasal adenocarcinoma
Hepadnaviruses:			
Rodent, avium	-	-	+
Cattle	-	-	-
HBV	-	-	hepatocellular adenocarcinoma

Class № 5. **Date_____**

Topic: Virologic diagnostics of diseases caused by hepatitis viruses, herpes- and adenoviruses.

Questions to study:	Medical Microbiology / F. H. Kayser, K. A. Bienz, J. Eckert , R. M. Zinkernagel // Thieme New York 333, Seventh Avenue, New York, NY 10001 USA.- 2005.:
1. Hepatitis viruses A, B, C, D, E, F, G, TTV and SEN. Taxonomy and characteristics, role in human pathology. Pathogenesis and immunity in hepatitis A, B, C. Laboratory diagnostics. Specific and non specific prophylaxis. 2. Herpesviruses. Taxonomy and family characteristics. HSV-1, HSV-2, properties, role in human pathology, pathogenesis, immunity, diagnostics, chemo and immunotherapy. HZV, properties, pathogenesis, immunity, diagnostics, prophylaxis. CMV: properties, pathogenesis. EBV features, role in human pathology. Pathogenesis, immunity, diagnostics. HHV6, HHV-7, HHV-8, role in human pathology. 3. Adenoviruses. Taxonomy and family characteristics. Human adenoviruses. Virions structures, pathogenesis, immunity, laboratory diagnostics	1. P. 437 – 438; 440; 429 – 434; 445 – 446. 2. P. 418 – 426; 3. P. 416 – 418.

Laboratory work

Laboratory exercises	**Laboratory report**
1. Performance of ELISA for VHC diagnostics.	1. The protocol is based on the commercial ELISA kit for VHC diagnostics "RecombiBest anti-HCV" by VectorBest, RF. The method reveals antibodies (IgG and IgM) to HCV antigens. Antibodies from patients serum bind to recombinant antigens adsorbed on the well of a plate. Specific immune complexes then detected by conjugate antibody-enzyme and respective enzymatic reaction. Colored product developed is measured by ELISA reader. 2. Reaction scheme: a) HCV antigens are adsorbed on the strip wells as follows: rows A, E – core rows B,F – NS3 rows C,G - NS4 rows D, H - NS5 b) put 100 mkl of control sera and samples according to the plate layout; c) close strip with adhesive tape and incubate for 1 hour at 37°C; d) wash wells 5 times; e) put 100 mkl of conjugate in each well; f) seal strip with tape and incubate for 30 min at 37°C; g) wash 5 times; h) put 100 mkl of substrate in each well; i) incubate for 30 min at 37°C; j) put 50 mkl of stop solution in each well; k) measure the plate by ELISA reader; l) evaluate results.

		1	2
CORE	A	Negative control	Serum 1
NS_3	B		
NS_4	C		
NS_5	D		
CORE	E	Positive control	Serum 2
NS_3	F		
NS_4	G		
NS_5	H		

ELISA protocol for VHC diagnostics

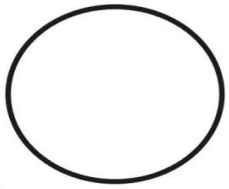
Date Technic name

Ag	Row	OD	Cut-off	Result
Core	**A**			
NS3	**B**			
NS4	**C**			
NS5	**D**			
Core	**E**			
NS3	**F**			
NS4	**G**			
NS5	**H**			

1. Test results validation:
   Negative control OD < 0,2
   Mean negative control OD =
   Mean positive control OD >0,8
   Mean positive control OD =
2. Cut-off level for each antigen:
   Cut-off (core-Ag) = NC ODO(core) + 0,2 =
   Cut-off (NS_3-Ag) = NC OD (NS3) + 0,2 =
   Cut-off (NS_4-Ag) = NC OD (NS4) + 0,2 =
   Cut-off (NS_5-Ag) = NC OD (NS5) + 0,2 =
3. Positivity index determination for each antigen:
   PI(core-Ag) = OD sample(core)/ Cut-off(core-Ag) =
   PI(NS_3-Ag) = OD sample (NS_3)/Cut-off(NS_3-Ag) =
   PI(NS_4-Ag) = OD sample (NS_3)/Cut-off(NS_4-Ag) =
   PI(NS_5-Ag) = OD sample (NS_3)/Cut-off(NS_5-Ag) =
4. Results evaluation:
   а) If PI less than 1, sample is considered negative;
   б) the results sre considered positive if IP exceeds 1 for:
      core-Ag
      any two antigens
   в) result is considered uncertain if IP exceeds 1 for one nonstructural protein only.

Doctor signature

Demonstration
1. Methods for HBs-Ag detection
2. CPE of adenoviruses.



Smear _____

_____

Stain_____

Tutor signature_____

Additional materials for the class № 5.

Methods for HbsAg detection in patient`s materials

Method	Virion number per ml of blood	Sensicity (ng\ml)
Precipitation test*	$1,0 \times 10^{11}$	2000
Counter immune electrophoresis (CIE)*	$2,0 \times 10^{10}$	400
Complement fixation test (CFT)*	$1,0 \times 10^{10}$	200
Reversed passive agglutination (RPAT)*	$1,0 \times 10^{9}$	20
Fast methods performed without appliances: Immunochromatography (ICA) Immunocomb (ELISA)	$1,0 \times 10^{9}$	 20 0,5
Radioimmune analysis (RIA)*	$0,5 \times 10^{6}$	0,05
Immunoenzyme analysis (ELISA)	$0,5 \times 10^{6}$	0,05
Immuno-chemiluminescence analysis (ICLA)	$0,5 \times 10^{6}$	0,05

* Not used at present

NB: In blood of patients with acute viral hepatitis B one can find large quantity of HbsAg. In blood of 80% non symptomatic carrier HbsAg concentration exceed 50 ng/ml; about 4% carriers (patients) have less than 0,5 ng/ml HbsAg in blood.

Clinical and epidemiological meaning of hepatitis A, B, C, D,E markers

Marker	Clinical and epidemiological meaning
VHA antigen (HAV-Ag)	Detection of HAV-Ag in children feces is an indication of infection danger for persons in the seat of infection (but is not diagnostic)
Total antibodies to VHA antigen (abHAV)	Indicate the current disease or past disease and is useful for vaccination need evaluation
IgM to VHA antigen (abHAV-IgM)	Acute hepatitis A marker
RNA of HAV (RNA-HAV)	Indicate HAV presence in material
HBs-Ag Surface HBV-antigen	Hepatitis B marker (both acute and chronic), require confirmation by total abHBc or abHBc-IgM. One of safety criteria in transfusiology. Screening in risk groups.
Total antibodies to HBs-Ag (abHBs)	Hepatitis B staging, prognosis, specific immunity control. Vaccination efficacy control. Epidemiological research. Favorable outcome marker.
Core HBV antigen (HBcAg)	Marker of HBV presence in hepatocyte (in acute or chronic hepatitis B).
IgG to HBV core antigen (abHBc)	Acute and chronic viral hepatitis B marker, carrier state marker, present or past HBV infection marker. Safety criterion in transfusiology.
IgM to HBV cor antigen (abHBc-IgM)	Acute hepatitis B or chronic hepatitis B exacerbation marker.
E-antigen of HBV (HBeAg)	The marker of HBV replication intencity and epidemiological danger of the patient. It is of importance for viral hepatitis differential diagnostics, prognosis of the disease outcome, evaluation of the vertical transmission risk.
Total antibodies to HBe-antigen (abHBe)	Staging of the disease, differential diagnostics. Marker of favorable disease outcome
HBV DNA	Marker of blood infectivity and active virus replication. Differential diagnostics between HBV or HBs-Ag carrier state
Total antibodies to HCV antigen (abHCV)	Hepatitis C marker. It is not suitable for disease staging.
IgM to HCV cor antigen (abHCc-IgM)	Acute hepatitis C marker (but sometimes can be found in chronic hepatitis C reactivation)
HCV RNA	Marker of blood infectivity
Total antibodies to HDV (abHD)	Hepatitis D marker. It is not suitable for disease staging.
IgM to HDV (abHD-IgM)	Acute hepatitis D marker
HDV RNA	Virus presence in blood
Total antibodies to HEV (abHEV)	Hepatitis E marker.

Virological diagnostics for herpes infection

A) Early diagnostics: morphological examination of damaged tissues and isolation of virus. Scrapings and smears from rash elements are used as a material.

- Smears are usually stained by Gimsa method or by hematoxylin-eosin. Giant cells formation and nuclear inclusion development are characteristic for herpes infection
- Smears can be stained with fluorescent antibodies (FAT). Herpes antigens may be found in multinucleated, giant and unchanged cells. The method allows to detect herpes infection in brain, spinal cord and other tissues (liver) in lethal cases.
- Virus can be isolated by
  - 12-days chicken embryo inoculation. Material is applied on allantois membrane. Embryo is incubated for 48 hours at 35 C. Allantois membrane damages are observed. Giant and multinucleated cells with nuclear inclusions are revealed by microscopy.
  - Cell culture inoculation. Typical CPE includes multinucleated cells formation with nuclear inclusions and round cell degeneration;
  - Suckling mice inoculation. Mice are infected in brain or in abdominal cavity. The disease appears in 3-4 days and kills animals;
  - Rabbits inoculation. Rabbits are infected on scarified cornea or in brain: specific keratitis or lethal encephalitis develops respectively.
- Identification of isolated viruses is performed by FAT or NT.

B) retrospective diagnostics

For serological diagnostics CFT or ELISA in paired sera are used.

Virological diagnostics for chicken pox

А**) Early diagnostics**: microscopy of material from lesions, viral antigens, DNA detection or virus isolation in cell culture.

- The best results are achieved by microscopy of material from fresh vesicular: multinucleated giant cells with nuclear inclusions are characteristic.
- For rapid identigication FAT method is usually used. Specific antigen can be revealed extracellularly as bright grains or intracellular.
- Virus can be isolated in cell culture. Characteristic CPE – the development of giant multinuclear cells or round-cell degeneration. Eosinofilic nuclear inclusions are often observed. Identification of isolated viruses is performed by FAT or NT.

Б) Retrospective diagnostics: specific antibodies are revealed in ELISA, CFT or NT in paired sera.

Virological diagnostics for EBV infection

1. Heterophilic antibodies detection — natural antibodies (IgM), which agglutinate erythrocytes of unrelated species (sheep, bull, horse etc). This phenomenon is found in approximately 90% EBV patients. Heterophilic antibodies sometimes present in blood of healthy persons in low titer.

а. Paul-Bunnell test (Hanganutziu-Deicher reaction) — standard method for infection mononucleosis diagnostics. It is based on sheep erythrocytes hemagglutination by patient`s serum. Diagnostic titer is 1:128—1:256. Heterophilic antibodies are found 3—4 week of the disease. **Paul-Bunnell test** are positive in leucosis, viral hepatitis, CMV infection, Burkitte lymphoma, rheumatoid arthritis, serum sickness. The antibodies titer does not reflect the severity of the disease.

б. The monospot test is a rapid test for infectious mononucleosis due to Epstein–Barr virus (EBV). The test is sensitive for heterophile antibodies which agglutinate horse erythrocytes. Commercially-available test kits are 70-92% sensitive and 96-100% specific. It will generally not be positive during the 4-6 week incubation period before the onset of symptoms. It will also not generally be positive after active infection has subsided, even though the virus persists in the same cells in the body for the rest of the carrier's life.

2. Serological diagnostics. Tests for heterophilic antibodies are relatively not sensitive and if negative can not exclude EBV infection. In this case other serological tests are useful:

а) ELISA for IgM and IgG to EBV capsid antigen. Its concentration reaches maximum in 2 weeks and diminishes during 2—3 months. IgM to EBV capsid antigen testifies for recent infection, IgG — infection in the past.

б) ELISA for antibodies to early EBV antigens. Its concentration reaches maximum in 2 weeks of the disease.

в) ELISA for antibodies to nuclear EBV antigen. They appear approximately in 4 weeks of the disease and persist lifelong.

Antibodies to EBV antigens

Antibodies		Period of the disease	Persistence	Specificity, %
Capsid antigens	IgM	Beginning	4-8 weeks	100
	IgG		lifelong	100
Early antigens	Anti-R	3-5 weeks	3-6 months	70
	Anti-D	2 weeks – 4 months	2 months – years	low
Nuclear antigen		3-4 weeks	lifelong	100

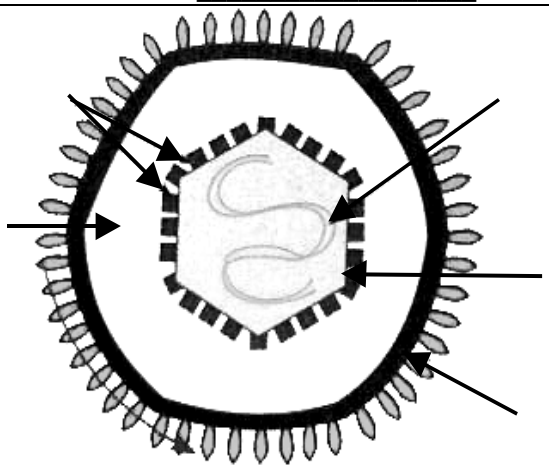
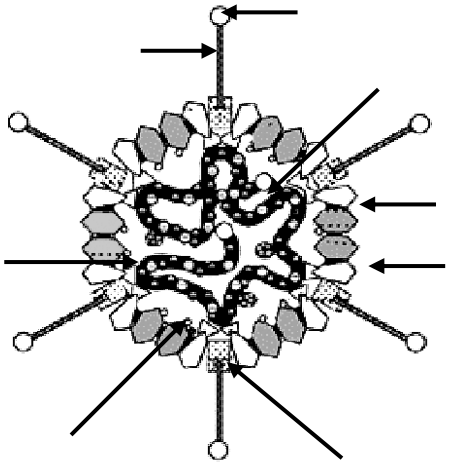
Virological diagnostics for adenovirus infection

1. Nasopharyngeal and conjunctival washes and scrapings, feces, urine, biopsy and autopsy are used as a material.
2. Fast methods include viral antigens and DNA detection in the material:
   usually FAT or ELISA in situ are used.
3. Virus isolation:

- different epithelial cell lines (HEK, HELA, A-549) are used. Characteristic CPE includes:
  - small cell degeneration with cell agglomeration (grape like);
  - cell rounding;
  - cytoplasmic and nuclear inclusions;
  - cells death;
- Virus identification is performed by NT, FAT, CFT;
- PCR;
- EM and IEM.

4. Retrospective diagnostics (for epidemiological purposes) includes ELISA, HIT, CFT in paired sera.

Write in virus family name, indicate respective structural elements of virion

The structure of _____virus.	
	1. Supercapsid 2. Glycoproteins 3. Icosahedral capsid 4. Capsomers 5. Tegument 6. DNA
The structure of _____virus.	
	1. Hexon 2. Penton 3. Fibers 4. Knob 5. Protein pVI 6. DNA 7. Primer pTp 8. Protease p23

Class № 6. **Date_____**

Concluding test: «Virology»

1. The systematic position and classification of viruses.
2. Forms of existence of viruses. The morphology and biochemical structures of virions. Prions.
3. Structure, properties and function of viral nucleic acids, proteins, lipids.
4. The interaction of viruses with susceptible cell. Strict parasitism, cytotropism of viruses. Cellular receptors for viruses.
5. The mechanisms of nonspecific and specific immunity to viral diseases. Interferon α, β, γ.
6. Types of viral infection of cells. Changes in host cells during viral infection. Cytopathic effect of viruses.
7. Inclusion in viral diseases. Nature, localization. Diagnostic value.
8. General principles of viral infections diagnostics. Methods for rapid diagnosis. Molecular and biological typing.
9. Cell culture, classification, characteristics. Cultivation of viruses in cell cultures. Preparation of the material, the contamination of culture. The methods of indication and identification of viruses.
10. Cultivation of viruses in chicken embryo. Methods of infection. Indication and identification of viruses.
11. Virus isolation in laboratory animals. Methods of infection of animals, display and identification of viruses.
12. Serological tests for viral infections. Hemagglutination inhibition test, hemadsorbtion inhibition test, neutralization test.
13. The etiology of acute respiratory viral diseases. Classification of influenza viruses. General characteristics. Structural and nonstructural proteins properties. The viral genome.
14. Antigenic structure of the influenza virus and its variability, the role in the epidemic and pandemic spread of influenza. Mechanisms of natural and acquired immunity.
15. Pathogenesis, specific and non-specific treatment and prevention of influenza.
16. Paramyxoviruses. The composition of the family. Parainfluenza, characterization, differentiation from influenza viruses. Mumps virus. Respiratory-syncytial virus.
17. Modern methods of laboratory diagnosis of influenza and parainfluenza.
18. Measles virus, morphology, cultural and antigenic properties. Pathogenesis and immunity in measles. Specific prevention of measles: vaccine, immunoglobulins.
19. The rabies virus, morphology, biological properties, viral inclusions. The pathogenesis of the disease. Laboratory diagnosis of rabies.
20. Epidemiology, specific and non-specific prophylaxis of rabies. Rabies vaccine and gamma globulin. Pasteur's work.
21. Retroviruses. Human immunodeficiency virus (HIV), the characteristic. Epidemiology, pathogenesis, laboratory diagnostics, prevention of HIV infection.
22. AIDS definition. The role of CD4 + and CD8 + T cells. AIDS-related illness.
23. Classification of hepatitis viruses. Characterization of hepatitis A virus pathogenesis, immunity, methods of prevention of hepatitis A.
24. Characterization of hepatitis B virus genome, the basic proteins. Pathogenesis, immunity, prevention, laboratory diagnosis of hepatitis B.
25. Hepatitis C, D, E. Characterization of viruses, epidemiology, pathogenesis of diseases.
26. Classification and characterization of environmental group “arboviruses”. Toga and flaviviruses. Importance in human pathology. Virological diagnostics for viral encephalitis.
27. Rubella virus. General characteristics. Role in the pathology. Prevention of rubella.
28. Bunyaviruses, general characteristics, diseases.
29. Picornaviruses, classification, general characteristics of the family.
30. Poliovirus, morphological and cultural characteristics, serological variants. Pathogenesis and laboratory diagnostics for poliomyelitis. Specific prevention of polio. Eradication of polio. Immunodeficiency - polio and flabby paralysis.
31. Coxsackie viruses and EKHO, characteristics. Role in human pathology. Principles of differentiation.
32. Rhinoviruses. Rotaviruses. General characteristics. Role in human pathology.
33. Adenoviruses, morphology, cultural, biological properties, serological classification. Pathogenesis, laboratory diagnostics of adenoviral infections.
34. Herpesviruses. Classification. General characteristics. Main proteins. Human disease caused by alpha-herpesviruses of the first and second serotypes.
35. General characteristics of varicella virus, cytomegalovirus, EBV. Mechanisms of pathogenesis. Laboratory diagnostics.
36. Theory of viral carcinogenesis. Oncogenic viruses. Cellular and viral oncogenes.
37. Bacterial viruses (bacteriophages), properties, classification. Interaction of bacteriophages with susceptible bacterial cell. Virulent and temperate phages. Lysogens.
38. The practical use of bacteriophages. Diagnostics, typing, treatment with bacteriophage preparations. Titration of bacteriophage