MINISTRY OF HEALTH CARE OF UKRAINE Kharkiv National Medical University D.P. Grynyov department of microbiology, virology and immunology

STANDARD PROTOCOLS

TO LABORATORY CLASSES IN SPECIAL VIROLOGY

for the II and III year English media students

student _____ year _____group

Surname_____

_____

Teacher_____

_____

Kharkiv 2018

Standard protocols to laboratory classes in special virology for the II and III year English media students of medical and dentistry faculties / M.M. Mishyna, N.I. Kovalenko, Yu.A. Mozgova, V.L. Tkachenko, T.M. Zamaziy, O.O. Vovk. – Kharkiv: KNMU, 2018. – 84 p.

Standard protocols are related to the program of Ministry of Health of Ukraine and is recommended to students of medical and dentistry faculties of high medical schools of III-IV accreditation level.

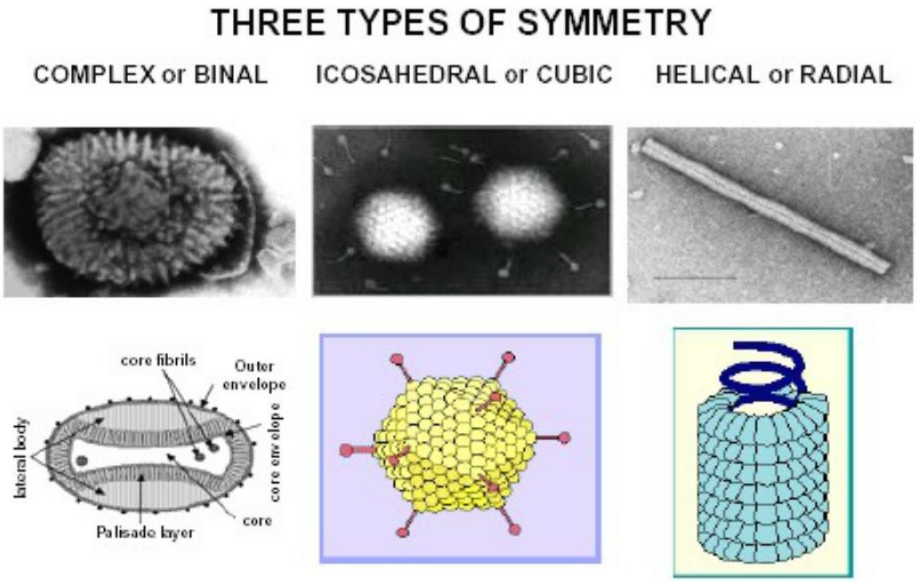
Standard protocols to laboratory classes in special virology includes demonstrative information according laboratory diagnosis of viral infections in humans. Standard protocols contain practical aspects together with theoretical information. They are structured and directed for individual work of students

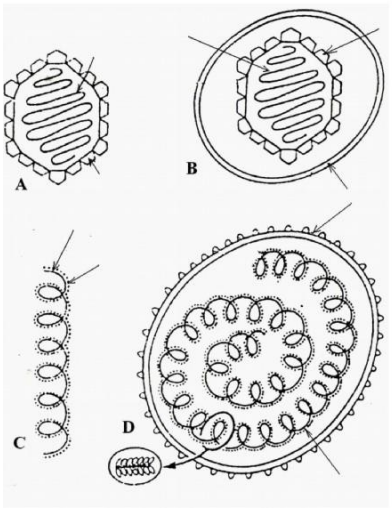
Special virology.

Protocol № 22

Theme: The main properties of viruses. Methods of laboratory diagnosis of viral diseases.

I. Morphology and the main properties of viruses.





A _____
B. _____

C. _____
D. _____

II. Methods of cultivation of viruses.

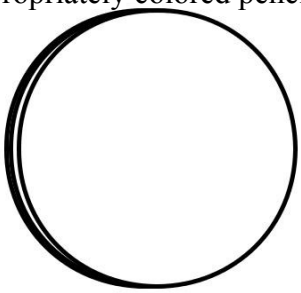
1). _____.
2). _____.
3). _____.

III. Cell cultures that are used for cultivation of viruses:

1. **Primary cell cultures** (Rhesus monkey kidney cell culture, Human amnion cell culture, Chick embryo fibroblast cell culture).
2. **Semi-continuous Cultures** (WI-38 – Human embryonic lung cell strain, HL-8 - Rhesus embryo cell strain).
3. **Continuous Cell Cultures** (HeLa – Human carcinoma of cervix cell line, HEP-2 – Human epitheliioma of larynx cell line, McCoy – Human synovial carcinoma cell line, KB – Human carcinoma of nasopharynx cell line, Vero – Vervet monkey kidney cell line, BHK-21 –Baby Hamster kidney cell line).

S u r n a m e _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ D a t e _ _ _ _ _ _ _ _ _ _ _

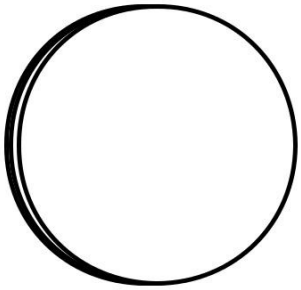
IV. Observe the smears below. Study cytopatic effects of viruses on cell culture. Using appropriately colored pencils draw the following cells.



Control of the tissue

Multinucleated gaint cell

Rounded cells

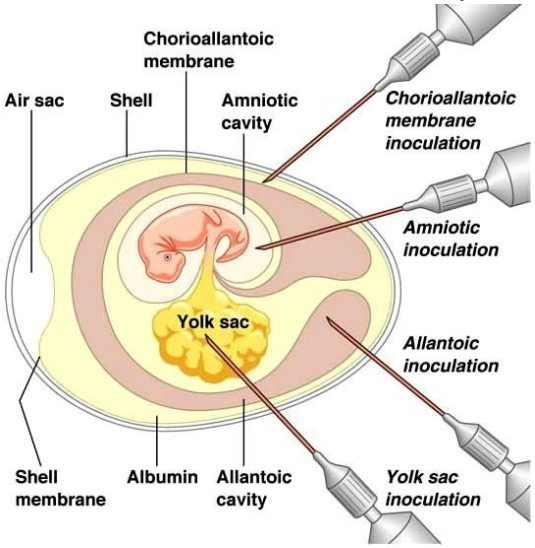


Lysis of cells

Intracytoplasmic inclusion

Babesh bodies (Lenz stain)

V. Study the structure and methods of inoculation of chicken embryo.



VI. Methods of indication and identification of viruses:

1. Recognition of viral growth in cell culture:
   a. Cytopathic effect (CPE);
   b. Hemadsorbtion.
2. Assay of viruses:
   a. Biological:
      1). Plaque assay;
      2). Transformation.
   b. Physical, biochemical:
      1). Hemagglutination;
      2). Immunological tests for proteins;
      3). Assay for nucleic acid (PCR);
      4). Enzymatic (reverse transcriptase for retroviruses).

For notes

For notes

For notes

Surname_____ Date_____

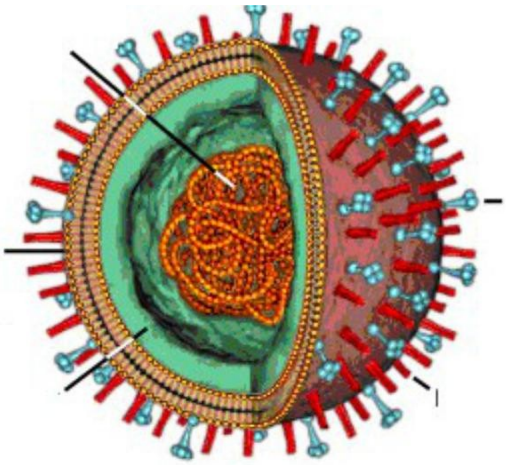
Protocol № 23, part 1

Theme: Laboratory diagnosis of influenza and acute respiratory viral infections.

I. Study classification of viruses - causative agents of respiratory viral infections.

Familly	Viruses
RNA viruses	
Orthomyxoviridae	Influenza viruses A, B and C
Paramyxoviridae	Parainfluenza, measles, mumps, and respiratory syncitial viruses
Picornaviridae	Rinoviruses, Cocsaky A and B viruses, ECHO viruses
Coronaviridae	Coronaviruses
Reoviridae	Reoviruses
DNA viruses	
Adenoviridae	Adenoviruses

II. Describe the scheme of influenza virus structure:



III. Study classification of influenza viruses

Type	Subtype	Years of circulation
A	H1N1	1918-1957
	H2N2	1957-1967
	H3N2	1968-
	H1N1	1977-
B	-	1940-
C	-	1949-

IV. Describe the following definitions of genetic variations of influenza viruses:

Antigenic drift _____

_____.

Antigenic shift _____

_____

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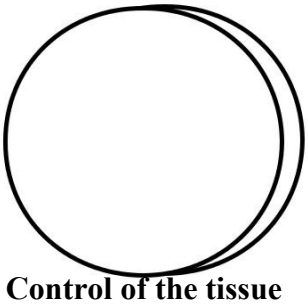
V. Observe the smears below. Using appropriately colored pencils draw the following cells.



Control of the tissue
(Hematoxylin and eosin stain)

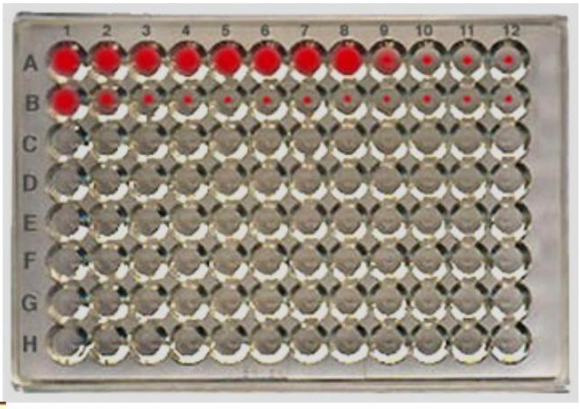
Intracytoplasmic inclusions of influenza virus
(Hematoxylin and eosin stain)

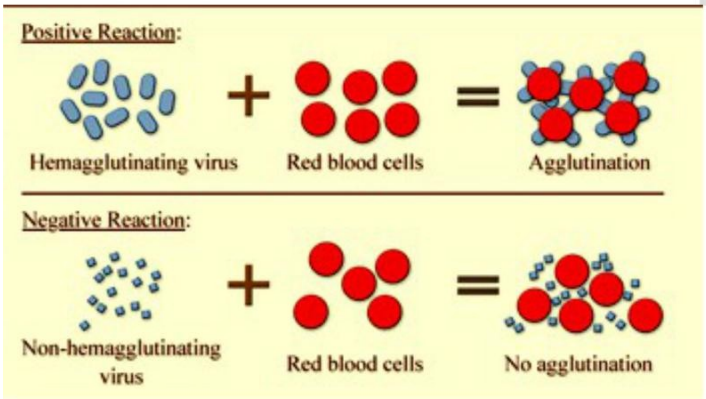
VI. Study of the mode of inoculation of chicken embryo with nasopharyngeal swab of influenza patient. Candling of chicken embryo and removing allantoic fluid.

VII. Performing of haemagglutination tests and interpretation the results.

Haemagglutination test is based on the ability of certain viruses to clump (agglutinate) red blood cells obtained from animals of definite species. Influenza and some other viruses with supercapsid membrane contain the surface antigen haemagglutinin responsible for the erythrocyte agglutination.

The HA test is performed on special plexiglass plates. A virus-containing specimen is double-diluted in 0.5 ml of isotonic saline. Half a millilitre of erythrocyte suspension thrice washed in isotonic saline is added into all test tubes, and 0.5 ml of erythrocyte suspension is mixed with an equal volume of virus-free isotonic sodium chloride solution, to be used as control. The mixture may be incubated at 37°, 20° or 4°C, depending on the properties of the tested virus.





Test results are assessed at 30-60 rain after complete erythrocyte sedimentation in the control, with the findings reading as follows:

(++++), intense and rapid erythrocyte agglutination with a star-like **,** marginally festooned sediment ("umbrella"); (++4-), residue of erythrocytes has clearings; (++), a less marked residue; (+), a floccular sediment surrounded with lumps of agglutinated erythrocytes, and (–), a markedly localized erythrocyte sediment ("rouleaus"), as in the control.

VIII. Study biological preparations for specific prophilaxis and therapy of influenza: inactivated virus, live and subunits vaccines, human immunoglobulin, human interferon.

IX. Study the scheme of laboratory diagnosis of influenza.
Specimen: the nose and the throat swab.
1. **Detection of antigen by immunofluorescence (IF) and ELISA**.
2. **The virus isolation** in cell culture or chicken eggs.
Identification of influenza strain and type: **hemagglutination inhibition (HAI) and hemadsorption inhibition (HadsI).**
3. Serology: **HAI, neutralization test (NtT), ELISA, CFT in pared sera.**

For notes

For notes

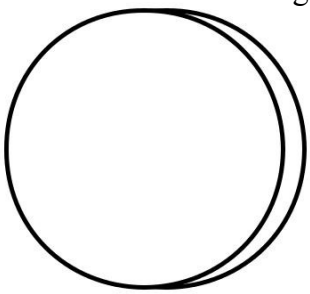
For notes

Surname_____ Date_____

Protocol № 23, part 2

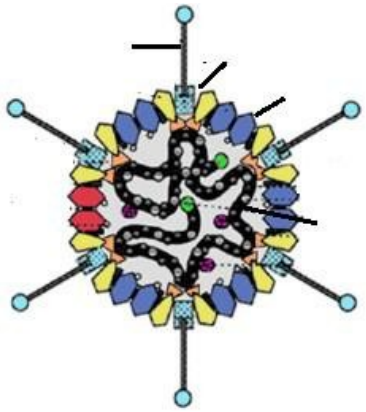
Theme: Laboratory diagnosis of adenoviruses infections.

I. Observe the smears below. Using appropriately colored pencils draw the following cells.



Control of the tissue **CPE of adenoviruses – rounding of cells**

II. Describe the scheme of adenonoviruses structure:



III. Describe the types of adenoviruses infections:

Productive infection _____.

Abortive infection: Virus interaction with a host cell can be blocked at many different steps, thus leading to an incomplete or abortive cycle.

Latency and persistence _____

_____.

Transformation: Soon after infection, the viral genome may be inserted into selective sites of the cellular genome. The initial steps of viral malignant transformation could involve insertional mutagenesis at a certain number of selective cellular sites. Cells from a number of rodent species and humans can be transformed in culture by adenoviruses. Tumorigenic potential has been attributed to the capacity of some adenoviruses (e.g., Ad12) to turn off the expression of genes of the major histocompatibility complex and thus to allow the transformed cells to overcome host defenses and grow into solid tumors.

IV. Study the scheme of laboratory diagnosis of adenoviruses infections.

1. **Detection of antigen** from pharyngeal and eye secretions and feces by:
   IF, ELISA and polymerase chain reaction.
2. **The virus isolation** in cell culture.
   Cytopathic effects (CPE) include swelling and rounding of cells.
   Identification: **HAI, CFT; type virus – by NtT.**
3. **Serology (rise in antibody titer)**: **HAI, NtT, CFT.**

For notes

For notes

For notes

Surname _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Date _ _ _ _ _ _ _ _ _ _

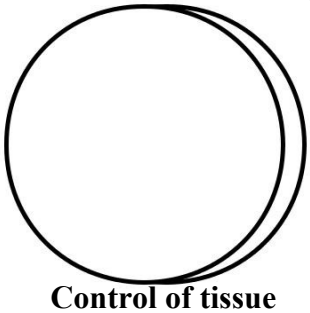
Protocol № 24

Theme: Laboratory diagnosis of enteroviruses infections.

I. Study classification of picornaviruses.

Genus	Viruses
Enterovirus	Polio viruses 1, 2, 3, Coxacki viruses A and B, ECHO viruses
Rhinovirus	Rinoviruses more then 100 types
Hepatovirus	Hepatitis A virus
Aphtovirus	Foot anf mouth disease cloven footed animals
Cardiovirus	Encephalomyocarditis viruses

II. Observe the smears below. Using appropriately colored pencils draw the following cells.



Control of tissue **CPE – lysis of cells**

III. Study mechanism of "color test".
IV. Study immunobiological preparations for diagnosis of enteroviruses infections:
а) typespecific polio sera 1-3 types for neutralization test and CFT;
б) typespecific Coxacki sera for neutralization test.
V. Study immunobiological preparations for prophylaxis of enteroviruses infections:
а) live Sebine's vaccine I-III types;
б) inactivated Solk's vaccine.
в) human immunoglobulin for treatment and prophylaxis of poliomyelitis.
VI. Study the scheme of laboratory diagnosis of enteroviruses infections.
Specimen: from feces, pharyngeal swabs, saliva, nasal aspirates, skin lesions, conjunctiva, cerebrospinal fluid, spinal cord, brain, heart, and blood.
1. Culture and isolation of viruses:

Virus	Tissue culture	Suckling mice
Polioviruses	+	-
Coxsackieviruses group A	-	+
Coxsackieviruses group B	±	+
Echoviruses	+	-

Identification: NtT, CFT, ELISA, HAIT.

2. **Serology** (four-fold or greater rise in neutralizing antibody titer between paired sera or high IgM titers to a single serotype): **NT; CFT; IF; ELISA; HAIT**.

For notes

For notes

For notes

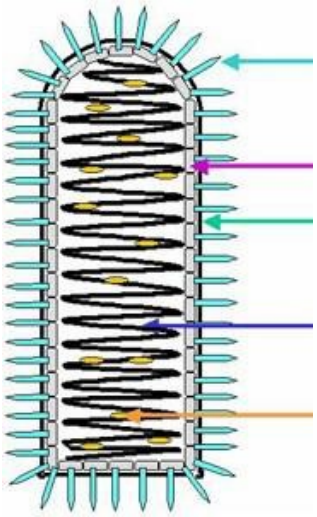
For notes

Surname_____ Date_____

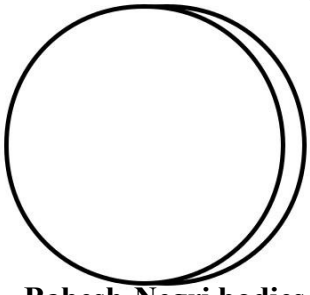
Protocol № 25

Theme: Laboratory diagnosis of rabies.

I. Describe the scheme of rabies virus structure:



II. Observe the smears below. Using appropriately colored pencils draw the following cells.



Babesh-Negri bodies (Lenz stain)

Babesh-Negri bodies (Turevich stain)

III. Study immunobiological preparations for prophylaxis of rabies:

a) inactivated rabies vaccine.

в) immunoglobulin.

IV. Study immunobiological preparations for diagnosis of rabies:

a) immuofluprescens serum for detection of rabies virus antigen in immunofluorescence .

V. Study the scheme of laboratory diagnosis of rabies.

1. **Detection of rabies antigen:** direct immunofluorescence:
   Antemortem: on a skin biopsy from the nape of the neck. Postmortem: on a brain biopsy.
2. **Detection of rabies virus-neutralizing antibody.**
3. **Cytology:** detection of Babesh-Negri bodies.
4. **Detection of viral RNA.**
5. **Isolation of virus** from saliva after inoculation of neuroblastoma cells or laboratory rodents; this is generally most successful during the first 2 to 3 weeks of illness.

For notes

For notes

For notes

Surname_____ Date_____

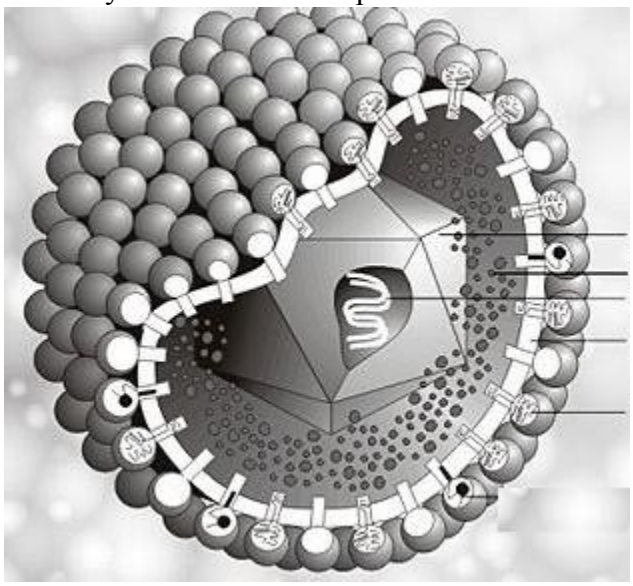
Protocol № 26

Theme: Laboratory diagnosis of herpes viruses infections.

I. Study classification of herpes viruses:

Taxonomy	Virus	Disease
Family Herpesviridae		
Subfamily Alphaherpesvirinae	Herpes simplex viruses 1 and 2 types	gingivostomatitis, ceratoconjunctivitis, encephalitis, genital herpes, herpes of newborn, cervix cancer
	Varizella-zoster (HV 3 type)	Varizella-zoster
Subfamily Betaherpesvirinae	Cytomegalovirus (HV 5 type)	Mononucleosolike syndrome, congenital cytomegalovirus infection, dessiminated infection in immunocompromised patients.
	Herpes virus 7 type	Chronic fatigue syndrom
Subfamily Gammaherpesvirinae	Epstain Barr virus (HV 4 type)	Infectious mononucleosis, Berkitt lymphoma, B-cell lymphoma, nasopharyngeal carcinoma
	Herpes virus 6 type	B-cell lymphoma
	Herpes virus 8 type	Kaposi sarcoma

II. Study the scheme of herpes virus structure:



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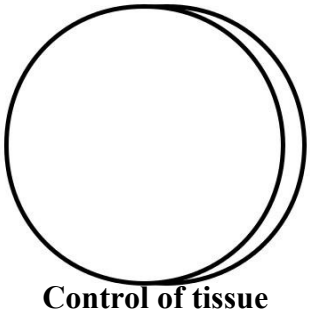
III. Observe the smears below. Using appropriately colored pencils draw the following cells.



Control of tissue

CPE of herpesviruses – multinucleated cells

IV. Study immunobiological preparations for specific prophylaxis of herpes viruses infections:

a) Live varicella-zoster vaccine.

в) Varizella-zoster immunoglobulin.

V. Study immunobiological preparations for therapy of herpes viruses infections

HSV-1 and HSV-2: Acyclovir, famcyclovir, valacyclovir, threefluridin.

Cytomegalovirus: Gancyclovir, foscarnet.

Varicella-zoster virus: Acyclovir, famcyclovir, valacyclovir.

VI. Study the scheme of laboratory diagnosis of herpes viruses infections.

1. Direct Detection:

Tzanck smear (multinucleated giant cells).

Immunofluorescence of skin scrappings.

PCR.

2. Virus Isolation on cell culture

Identification: IF, ELISA

3. **Serology:** (IgG in paired acute and convalescent sera; IgM tests are likely to prove invaluable in determining the nature of congenital varicella infections): ELISA, IF, CFT.

For notes

For notes

For notes

For notes

For notes

For notes

Surname_____ Date_____

Protocol № 27, part 1

Theme: Laboratory diagnosis of arboviruses infections.

I. Study classification of arboviruses:

Familly	Disease		
	Encephalitisis	Fevers	Hemorragic fevers
Togaviridae **(Alphaviruses)**	Western equine encephalitis	Chikungunya	Chikungunya
	Eastern equine encephalitis	Sindbis	
	Venezuelan equine encephalitis	Rose River Fever	
Flaviviridae	Japanese encephalitis	Dengue	Dengue
	Tick-borne encephalitis	West Nile Fever	Yellow fever
	Murray Valley encephalitis		Kyassanur Forest disease
			Omsk Fever
Bunyaviridae	Californian encephalitis	Rift valley Fever	Congo-cremean
Reoviridae		Coloradic tick Fever	
Rhabdoviridae		Vesicular stomatitis	

II. Study immunobiological preparations for specific prophylaxis of arboviruses encephalitisis: a) killed culture vaccine against tick born encephalitis,
в) immunoglobulin against tick born encephalitis,
c) live and killed vaccines against japanese aencephalitis,
d) immunogloubulin against japanese encephalitis.

III. Study the scheme of laboratory diagnosis of tick born encephalitis.
Specimen: blood or from brain, liver, and other organs postmortem.

1. **Isolation of virus** during the viremic phase in mammalian or insect cell cultures.
2. **Serology:** (demonstration the presence of IgM antibody or a fourfold rise in titer of IgG antibody between acute- and convalescent-phase serum): HIT, ELISA, CFT, immunefluorescence.
3. **Detection of RNA by RT-PCR**.

For notes

For notes

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S u r n a m e _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ D a t e _ _ _ _ _ _ _ _ _ _

Protocol № 27, part 2

Theme: Laboratory diagnosis of hemorrhagic fevers.

I. Study classification of hemorrhagic fevers viruses:

Group	Familly	Disease
Tick born	***Bunyaviridae***	Congo-cremean hemorragic fever
	Flaviviridae	Omsk Hemorragic fever
	Flaviviridae	Kyassanur Forest disease
Mosquito	***Flaviviridae***	Yellow fever
	Flaviviridae	Dengue
	Togaviridae	Chikungunya
Contact	***Bunyaviridae***	Hemorragic fever with renal syndrome
	Arenaviridae	Lassa hemorragic fever
	Filoviridae	Ebola hemorragic fever
	Filoviridae	Marburg hemorragic fever

II. Study immunobiological preparations for specific prophylaxis of hemorragic fevers:

Congo Cremean hemorragic fever:

a) specific immunoglobulin from blood of reconvalescents or immunized people;

b) killed vaccine.

Yellow fever:

a) live vaccine 17 D and «Dacar».

Dengue hemorragic fever:

a) specific immunoglobulin from blood of reconvalescents.

Hemorragic fever with renal syndrome:

a) human immunoglobulin;

b) killed culture vaccine K-27.

VI. Study the scheme of laboratory diagnosis of hemorrhagic fevers.

Specimen: blood, urine, or throat washings

1. Isolation of virus during the viremic phase in mammalian or insect cell cultures**.**

2. Serology: (demonstration the presence of (IgM) or a fourfold rise in titer of IgG between acute- and convalescent-phase sera): CFT, HAI, ELISA, immunefluorescence.

3. Detection of RNA by RT-PCR.

For notes

For notes

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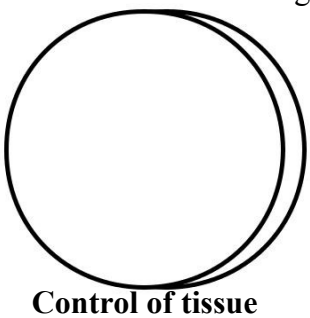
Surname_____ Date_____

Protocol № 28

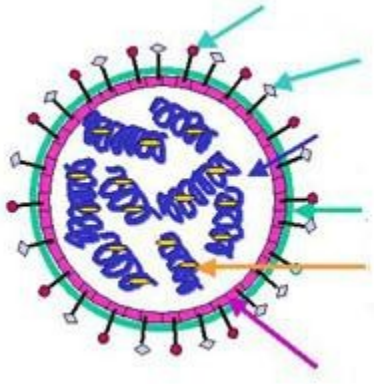
Theme: Paramyxoviruses (morbilliviruses). Laboratory diagnosis of measles and rubella.

I. Observe the smears below. Using appropriately colored pencils draw the following cells.



Control of tissue **CPE of measles virus – muclinucleated cells**

II. Describe the scheme of measles virus structure:



III. Study immunobiological preparations for specific prophylaxis of measles and rubella: a) live measles vaccine;
b) human immunoglobulin;
c) live vaccine against rubella;
d) live vaccine against measles, rubella and parotitis.

IV. Sudy the scheme of laboratory diagnosis of **measles**.
1. Detection of antigen from nasopharyngeal aspirates and throat swab by IF.
2. The virus isolation in cell culture.
CPE: giant multinucleated cells formation.
Identification: HAI, IF, NtT.
3. Serology (detection of antibodies): HAI, NtT, CFT.

V. Sudy the scheme of laboratory diagnosis of **rubella**.
1. **Serology:** HAI, CFT, NtT, IF **(r**ising Ig G antibody titres - 4-fold or greater). Presence of rubella-specific IgM: ELISA.
2. **Isolation the virus in cell cultures** from respiratory tract secretions and, in infants with congenital infection, from urine, cerebrospinal fluid, and blood.
Identification: viral interference, HAI, IF, ELISA.

For notes

For notes

For notes

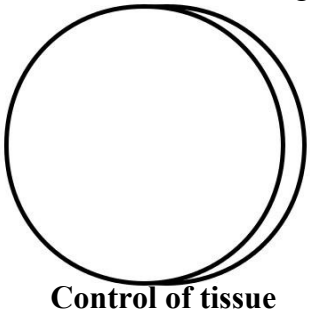
For notes

Surname _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Date _ _ _ _ _ _ _ _ _ _ _

Protocol № 29

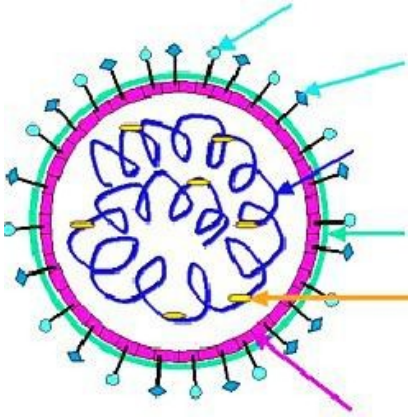
Theme: Paramyxoviruses. Laboratory diagnosis of mumps and parainfluenza.

I. Observe the smears below. Using appropriately colored pencils draw the following cells.



Control of tissue **CPE – inclusion body**

II. Describe of paramyxoviruses structure:



III. Study immunobiological preparations for diagnosis of mumps and parainfluenza:
a) typespecific parainfluenza sera for CFT and HAI
b) immuofluprescent serum for detection of parainfluenza virus in immunofluorescence.

IV. Study immunobiological preparations for specific prophylaxis of mumps and parainfluenza:
a) live mumps vaccine;
b) live vaccine against measles, parotitis and rubella (MMR).

V. Study the scheme of laboratory diagnosis of **mumps**.
1. The virus isolation from the saliva, liquor or urine in cell culture (or chicken eggs).
CPE: giant multinucleated cells formation**.**
Identification: HAI, NtT, IF, CFT.
2. Serology: HAI, NtT, ELISA, CFT (demonstrating IgM in the first serum and detecting IgG rise in paired sera).

VI. Study the scheme of laboratory diagnosis of **parainfluenza**.
1. Detection of antigen from nasopharyngeal aspirates and throat swab by IF and PCR.
2. The virus isolation in cell culture.
Indication: Haemadsorption of erythrocytes on the surface of cells infected with virus.
Identification: HadsI, HAI, NtT, CFT.
3. **Serology**: NtT, ELISA, CFT, HAI (detection of rise in titer of IgG in pared sera).

For notes

For notes

For notes

For notes

Surname _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Date _ _ _ _ _ _ _ _ _ _

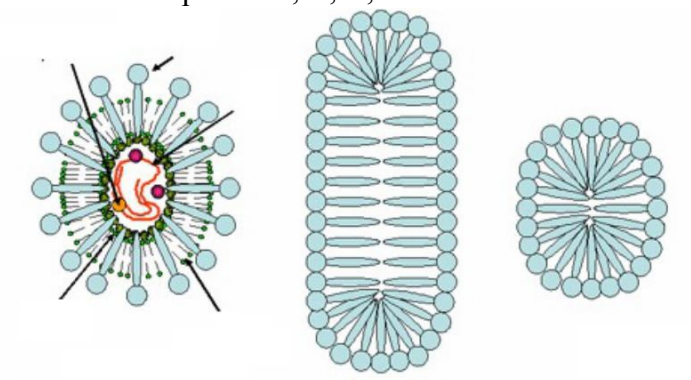
Protocol № 30

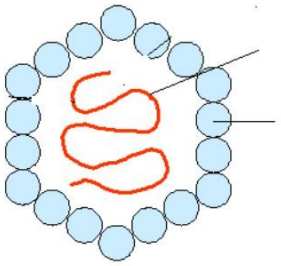
Theme: Laboratory diagnosis of viral hepatitis.

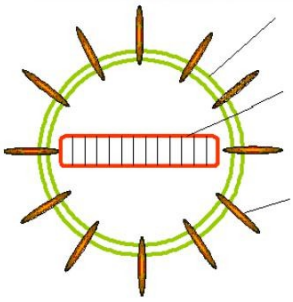
I. Study classification of hepatitis viruses:

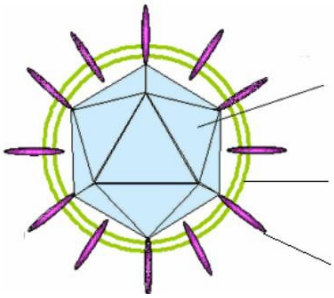
Virus	Familly	Nucleic acid	Envelope
Hepatitis virus A	Picornaviridae	RNA	No
Hepatitis virus B	Hepadnaviridae	DNA	Yesь
Hepatitis virus C	Flaviviridae	RNA	Yes
Hepatitis virus G	Flaviviridae	RNA	Yesь
Hepatitis virus D	Genus Deltavirus	RNA	Yesь
Hepatitis virus E	Hepeviridae	RNA	No
Hepatitis virus TTV	Circinoviridae	DNA	No
Hepatitis virus SEN	Circoviridae	DNA	No

II. Describe the scheme of hepatitis A, B, C, and D viruses structure:









Hepatitis A vrus Hepatitis D virus Hepatitis C virus

III. Study immunobiological preparations for specific prophylaxis of viral hepatitsis: a) killed vaccine against hepatitis A;
b) recombinated vaccine against hepatitis B;
c) human immunoglobulin.

IV. Serological markers of hepatitis B.

Markers	Disease state			Healthy state	
	Acute	Chronic	Late acute	Resolved	Vaccinared

Anti HBe	-	+	±	+	-
Anti HBc	-	-	±	+	-
Anti HBc Ig M	+	-	-	-	-
Anti HBs	-	-	-	+	+
HBeAg	+	+	-	-	-
HBs Ag	+	+	+	-	-
DNA	+	+	+		

For notes

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Surname_____ Date_____

Protocol № 31

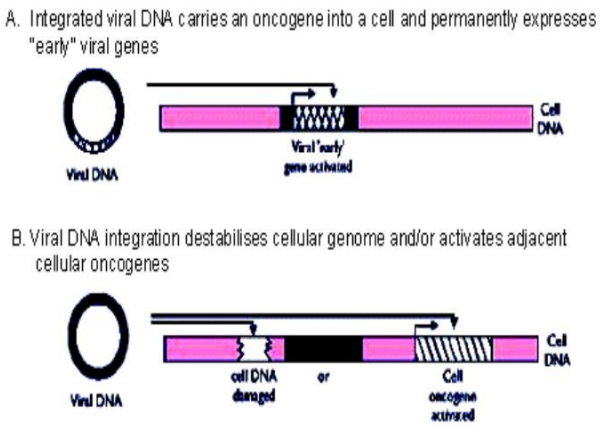
Theme: Oncogenic viruses. Antitumor immunity.

I. Study classification of oncogenic viruses:

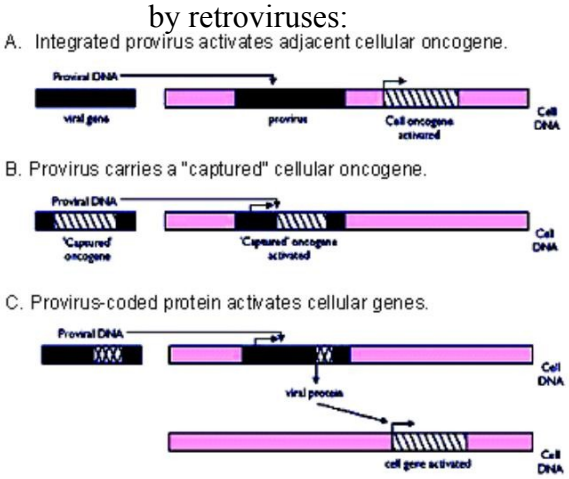
Taxomony	Virus	Disease
DNA viruses		
Familly Herpesviridae		
Subfamilly Alphaherpesvirinae	Herpes simplex viruses type 2	Cervix cancer
Subfamilly Betaherpesvirinae	Cytomegalovirus	Transformation of human cells in vitro
Subfamilly Gammaherpesvirinae	Epstein-Barr virus	Berkitt's lymphoma, B-cell lymphoma, nasopharyngeal carcinoma
	Herpes virus type 6	B-cell lymphoma
	Herpes virus type 8	Kaposi sarcoma
Familly Hepadnaviridae	Hepatitis B virus	Hepatocellular carcinoma
Familly Papovaviridae	Papilomaviruses	warts, condilomas, cervix carcinoma, carcinoma of larynx
Familly Adenoviridae	Adenoviruses	Nondefertiated tumors of rodents
***Семейство* Poxviridae**	Contagious mollusc virus, mankey pox virus	Benign conguctive tissue tumors of rodents and mankeys
RNA viruses		
Familly Flaviviridae	Hepatitis C virus	Hepatocellular carcinoma
Familly Retroviridae	Human lymphotropic viruses HTLV-1, HTLV-2	T-cell leucosis, hairlike leucosis

II. Mechanism of viral cancerogenesis:

by DNA viruses:



by retroviruses:



III. Antitumor immunity:

Humoral factor: complement, antibodies.

Cellular factors: dendrites cells, neutrophils, macrophages, NK cells, T cells.

Cytokines: transforming growth, factor beta (TGFß), cytokines (IL-10, IL-12), γ-interferon.

For notes

For notes

Surname_____ Date_____

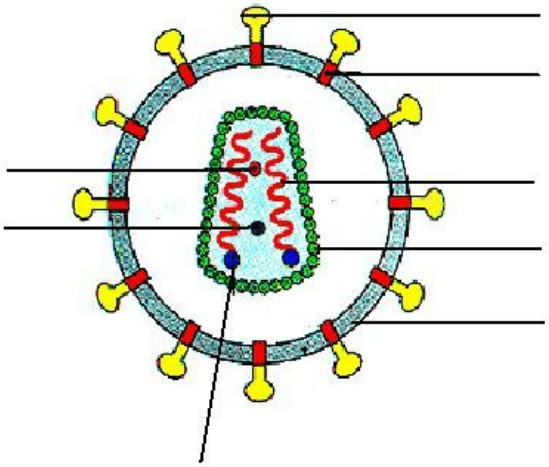
Protocol № 32

Theme: Retroviruses. ***Human immunodeficiency virus.***

I. Study classification of retroviruses:

Genus	Viruses
Alpharetrovirus	Rous sarcoma virus
Betaretrovirus	Mouse mammary tumor virus
Gammaretrovirus	Monkey and mice sarcoma and leukemia virus
Deltaretrovirus	Human T-lymphotropic viruses HTLV-1, HTLV-2, HTLV-5
Epsilonretrovirus	Skin sarcoma virus
Lentivirus	**Human immunodeficiency viruses HIV-1, HIV-2**
Spumavirus	Human foamy virus

II. Describe the scheme of HIV structure:



III. Study drugs for therapy of HIV infection:

Nucleoside-Analog Reverse Transcriptase Inhibitors (NRTI) inhibit viral RNA-dependent DNA polymerase and are incorporated into viral DNA (they are chain-terminating drugs).

- Zidovudine (ZDV, Retrovir) first approved in 1987
- Stavudine
- Lamivudine etc.

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) inhibit HIV replication directly by binding non-competitively to reverse transcriptase.

- Nevirapine
- Delavirdine

Protease Inhibitors are specific for the HIV-1 protease and competitively inhibit the enzyme, preventing the maturation of virions capable of infecting other cells.

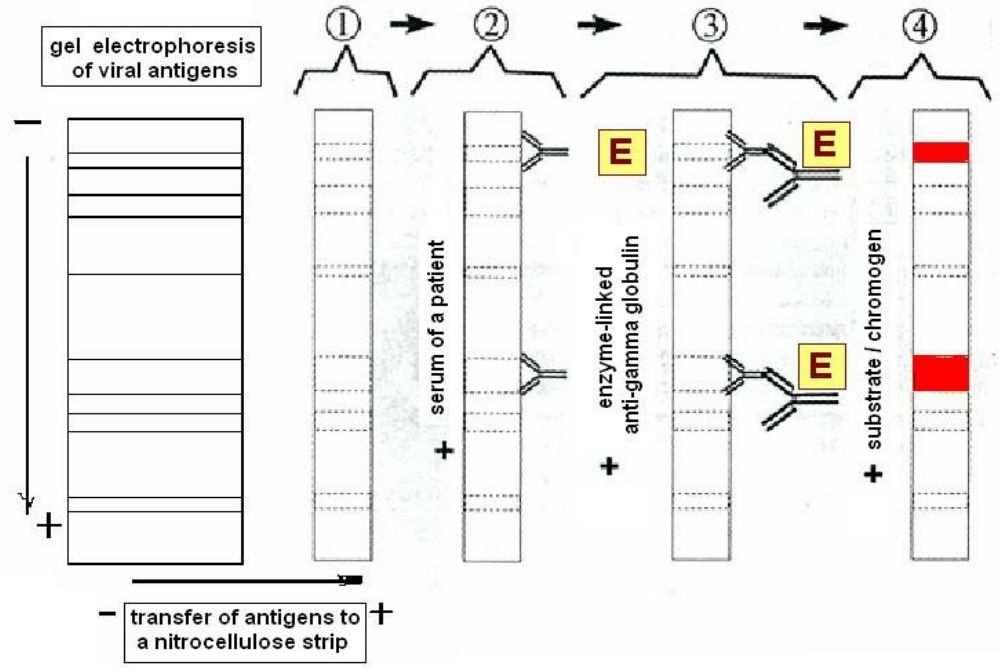
- Saquinavir (Invirase) first approved in 1995
- Ritonavir
- Indinavir etc.

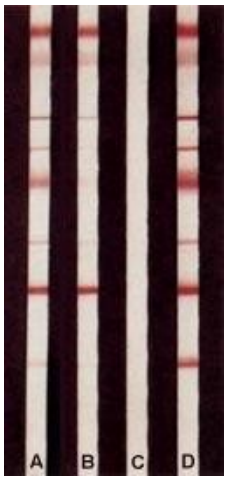
S u r n a m e _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ D a t e _ _ _ _ _ _ _ _ _ _ _

IV. Study the scheme of laboratory diagnosis of HIV-infection.

1. **Antigen detection:** ELISA (core antigen p24). In the fist few weeks after infection and in the terminal phase, the test is uniformly positive.
2. **Virus isolation:** from the peripheral lymphocytes by co-cultivation of the patient's lymphocytes with uninfected lymphocytes in the presence of interleukin-2. It is not suitable as a routine diagnostic test.
3. **Polymerase chain reaction.** The gold standard for diagnosis in all stages of HIV infection. It becomes necessary particularly in the course of treatment.
4. **Antibody detection:** IgM antibodies appear in about 4-6 weeks to months after infection, to be followed by IgG antibodies.
   - **Screening test**: ELISA assay.
   - **Confirmatory test**: Western blot.

V. Study principle of Western blot to diagnosis of HIV-infection:





Strip A – Positive control
Strip B – Light positive control
Strip C – Negative control
Strip D – Positive specimen
(antibodies against HIV-1 are detected)

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QUESTIONS TO EXAMINATION IN MICROBIOLOGY, IMMUNOLOGY AND VIROLOGY

SPECIAL VIROLOGY

1. Morphology and ultrastructure of viruses. Types of symmetries. Chemical composition, functions of components of viruses.
2. Principles of classification of viruses. Main families of viruses of man and animals.
3. Factors of antiviral immunity: humoral and cell-mediated immunity.
4. Nonspecific factors of protection of the macroorganism against viral agents, their characteristic. Interferons, mechanism of their action, interferonogens.
5. Methods for cultivation of viruses and their identification.
6. Use of cell cultures in virology, their classification. Types of interaction of viruses and cells. Stages of productive interaction. Cytopathic effect of viruses on cell cultures.
7. Methods for revealing viruses. Reactions of viral hemagglutination and hemadsorption. Mechanism, practical use and diagnostic value.
8. The reaction of virus neutralization, its mechanism, principles of use, diagnostic value.
9. The complement fixation test, its mechanism, principles of use, diagnostic value.
10. Orthomyxoviruses. Biological properties, antigenic structure, classification of influenza viruses. Pathogenesis and immunity in influenza. Role of specific and nonspecific mechanisms in influenza immunity. Specific prophylaxis and therapy. Methods of laboratory diagnosis of influenza.
11. Adenoviruses. Antigenic structure. Cultivation. Pathogenesis and laboratory diagnosis of adenoviral infections. Immunity, specific prophylaxis.
12. The family of Picornaviridae, its general characteristic. Biological properties. Antigens. Significance in pathology of man.
13. Viruses of poliomyelitis, its characteristics and classification. Pathogenesis and immunity. Laboratory diagnosis, specific prophylaxis.
14. The genus of enteroviruses, their general characteristics. The viruses of Coxsackie and ECHO. Their biological properties and the role in pathology of man. Diagnosis of enteroviral infections.

15. Rhabdoviruses. The virus of rabies, its structure. Pathogenesis of the disease. Specific prophylaxis. Laboratory diagnosis. Differentiation of the fixed and wild viruses of rabies.

16. The family of paramyxoviruses, general characteristics. The viruses of parainfluenza, measles and epidemic parotitis, their biological properties. Their role in pathology of man. Laboratory diagnosis of the diseases. Specific prophylaxis.

17. Rubella virus, its biological properties. Pathogenesis of the disease. Specific prophylaxis. Laboratory diagnosis.

18. Herpesviruses, their classification and biological properties. Significance in pathology of man. Laboratory diagnosis of the diseases.

19. Arboviruses, their classification and biological properties. Significance in pathology of man. Laboratory diagnosis of the diseases. Specific prophilaxis.

20. Hepatitis viruses, their general characteristics. Significance in pathology of man. Laboratory diagnosis of viral hepatitis. Specific prophilaxis of viral hepatitis.

21. 0ncogenic viruses, their classification. Viral-genetic theory of the origin of tumors proposed by Zilber. Retroviruses, their biological properties. Mechanism of the viral cancerogenesis. Anti-tumor immunity.

22. Human immunodeficiency virus. Structure, antigens. Pathogenesis, mechanism of immunodeficiency. Clinical stages. Laboratory diagnosis. Treatment.

23. Prions, properties, functions of normal prions, proliferation. Prion diseases, transmission, pathogenesis, laboratory diagnosis.

28. Haemophilus influenzae, its morphological, cultural and antigenic properties. Microbiological diagnosis and specific prophylaxis of haemophilus infection.

29. Opportunistic microorganisms, their biological properties and role in pathology of man. Pseudomonas aeruginosa and Proteus. Etiological role in purulent processes. Significance in etiology of hospital infections. Microbiological diagnosis.

30. The family of Spirochaetaceae: Leptospira and Borrelia, their characteristics and classification. Pathogenesis of the disease, immunity, microbiological diagnosis of the diseases. Specific prophylaxis, therapy.

31. The causative agent of syphilis. Morphological and cultural characteristic. Pathogenesis and immunity. Microbiological diagnosis and specific therapy of syphilis.

32. Pathogenic protozoa, biological properties. Classification, role in pathology of man.

33. Toxoplasma gondii, their morphology, peculiarities of cultivation. Pathogenesis of the diseases. Microbiological diagnosis. Specific prophylaxis.

34. Plasmodia malariae, their characteristics. Pathogenesis of malaria. Microbiological diagnosis. Specific prophylaxis, therapy.

35. Entamoeba histolytica, its morphology. Pathogenesis of the diseases. Microbiological diagnosis. Therapy.

36. Trichomonas vagimalis, its morphology. Pathogenesis of the diseases. Microbiological diagnosis. Therapy.

37. Giardia lamblia, its morphology. Pathogenesis of the diseases. Microbiological diagnosis. Therapy.

38. Leishmania, species, morphology. Pathogenesis of the diseases. Microbiological diagnosis. Therapy.

39. Mycoplasmatales, classification. Biological properties, methods of cultivation. Role in pathology of man. Microbiological diagnosis of mycoplasmoses.

40. Chlamydia, classification. Biological properties, methods of cultivation. Role in pathology of man. Microbiological diagnosis of chlamydiases.

41. Rickettsiae, their biological properties. Classification. Rickettsiae as causative agents of diseases in man. Pathogenesis of the disease, laboratory diagnosis, specific prophylaxis.

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