## Laboratory diagnostics of Orthomyxoviruses, Paramyxoviruses, Respiratory syncytial infections and Rubella, Morbilliviruses infections.

| Characteristic of Orthomyxoviruses family | The genome of orthomyxovirus is single-stranded negative sense and segmented RNA. The virion has envelop, helical nucleocapsid, and contains RNA dependend RNA polymerase. The envelope contains two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and is internally lined by the matrix (M1) and membrane (M2) proteins. The HA has several functions: it is the viral attachment protein, binding to sialic acid on epithelial cell surface receptors; it promotes fusion of the envelope to the cell membrane; it hemagglutinates (binds and aggregates) human, chicken, and guinea pig red blood cells; and it elicits the protective antibody response. |
| Antigenic Variations of Influenzaviruses | Influenza viruses have a propensity of undergoing antigenic variations. Immunity acquired through infection by one virus is frequently insufficient to prevent infection by a variant. This antigenic variation is the main reason why it is difficult to prevent influenza through immunization and why recurrences of influenza in the same person are common. Key points to understand about antigenic variation are:  
1. Major antigenic change (antigenic shift) is due to the replacement of one H (or N) gene by another.  
2. Minor antigenic change (antigenic drift) is due to mutation of the H (or N) gene of types A and B viruses. |
| Definition of Antigenic drift | A mechanism for variation by viruses that involves the accumulation of mutations within the antibody-binding sites so that the resulting viruses cannot be inhibited well by antibodies against previous strains making it easier for them to spread throughout a partially immune population. Antigenic drift occurs in both influenza A and influenza B viruses. |
| Definition of Antigenic shift | Sudden shifts in the antigenicity of a virus result from the recombination of the genomes of two viral strains. Antigenic shift is seen only with influenza A viruses. It results usually from the replacement of the hemagglutinin (the viral attachment protein that also mediates the entry of the virus into the cell) with a novel subtype that has not been present in human influenza viruses for a long time. The source of these new genes is the large reservoir of influenza viruses in waterfowl. The consequences of the introduction of a new hemagglutinin into human viruses are usually a pandemic, or a worldwide epidemic. |
| A source of infection is at a flu | 1. Sick people  (animals is a reservoir of infection)  
2. Viruscarrae |
| Ways of transmission of influenza | Aeroborn |
| Basic stages of pathogenesis of influenza | An infection is through nose and pharingis  
Penetration of virus is in the cages of cylinder epithelium by neurominidase  
Primary reproduction of virus is in the epithelium of respiratory tracts  
Viremia |
<table>
<thead>
<tr>
<th>General intoxication syndrome</th>
<th>↓</th>
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</thead>
<tbody>
<tr>
<td>Defeat of lymphocytes</td>
<td>↓</td>
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<tr>
<td>Second immunodeficiencies, complication</td>
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</table>

| Principle the laboratory diagnosis of influenza | Study the agent in material from patient. |
| Method for laboratory diagnosis of influenza | Indication of the virus |
| | Indication of the specific changes in the body |

| Method for laboratory diagnosis of influenza | Immunofluorescent microscopy (express-method). |
| | Virological method. |
| | Serological method. |

| Express-method for diagnosis of influenza | An exposure of virus (to the antigen) is in experimental material by IFT (direct and indirect) and ELISA. It is possible to find out in material the genome of viruses by PCR. |

| Necessary ingredients for the accumulation of virus in the virological method | Experimental material |
| | Chicken embryo or cell culture in an media 199 |

| An indication of influenza viruses in the virology method of diagnostics | The indication of the virus is conducted depending on a laboratory model (after death, after clinical and patomorphologic changes, after CPE, HAT, HAdaT) |

| Ingredients for identification of influenza virus in HAIT | Isolated from patient virus |
| | Specific immune serum |
| | Erythrocytes |

| Ingredients for determination of antibodies against the influenza viruses in HAIT (to the serodiagnostic flu) | Serum from patient |
| | Specific viruses diagnosticum |
| | Erythrocytes |

| A factor of confirmation of diagnosis of flu is by serological method | Incrise of titer of antibodies no less than in 4 times |

| Basic mechanisms of immunity are at a flu | Antibody – antihemagglutinin, antineurominidase |
| | Immunoglobulin A |
| | INFα |
| | Termolabil inhibitor |
| | Natural T-killer, macrophage |
| | Anti-influenza immunity is protective and strained, but very specific (type-, subtype- and even variantspecific) |

| Facilities of creation of active immunity are at a flu | Living vaccines of different types |
| | Chemical vaccines (recombination) |
| | Inactivated vaccines |

| Facilities of creation of passive immunity are at a flu | Antiinfluenza serum |
| | Gamma-globulin antiinfluenza |

| Preparations are for the unspecific prophylaxis of flu | Interferons |

| Taxonomic position of the parainfluenza virus | Family Paramyxoviridae |
| | Serotypes 1 and 3 behave to the sort of Respirovirus, and serotypes 2, 4a, 4b - Rubulavirus |

<p>| Parainfluenza | It is acute respiratory virus infection characterized the overwhelming |</p>
<table>
<thead>
<tr>
<th><strong>Basic structural components of parainfluenza virus</strong></th>
<th>defeat of overhead respiratory tracts and moderate intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RNA</td>
<td></td>
</tr>
<tr>
<td>2. Capsid</td>
<td></td>
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<tr>
<td>3. External shell of lipide-carbohydrate protein membrane</td>
<td></td>
</tr>
<tr>
<td><strong>Type of the virus</strong></td>
<td>The parainfluenza virus distinguish on 4 basic serotypes of viruses depending of the the HN, NP, F antigens</td>
</tr>
<tr>
<td><strong>Resistance of parainfluenza viruses in an external environment</strong></td>
<td>Quickly perish under act of different factors: temperature, acids, alkali, UFV and in, sensible to of tables disinfections.</td>
</tr>
<tr>
<td><strong>Epidemiology of parainfluenza</strong></td>
<td>Sours of infections is sick human Way of transmission is aeroborn, rarely contact</td>
</tr>
<tr>
<td><strong>Basic stages of pathogenesis of parainfluenza</strong></td>
<td>Penetration of virus through the mucuses membrane to the nose, gullet, larinx</td>
</tr>
<tr>
<td></td>
<td>Reproduction of virus is in the cells of superficial epithelium overhead respiratory tracts</td>
</tr>
<tr>
<td></td>
<td>Virusemia, toxinemia</td>
</tr>
<tr>
<td></td>
<td>Defeat of larynx, trachea</td>
</tr>
<tr>
<td></td>
<td>Second immunodeficient that assists development of bacterial complications</td>
</tr>
<tr>
<td><strong>Methods of laboratory diagnostics of parainfluenza</strong></td>
<td>1. Express-method (IFT, PCR)</td>
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<tr>
<td></td>
<td>2. Virological method of diagnostics</td>
</tr>
<tr>
<td></td>
<td>3. Serological method of diagnostics (HAIT, CFT, NT)</td>
</tr>
<tr>
<td><strong>Features of postinfection immunity</strong></td>
<td>Of short duration and nonstrained, typospecific, reinfection is possible.</td>
</tr>
<tr>
<td><strong>Preparations for specific prophylaxis and treatment of parainfluenza</strong></td>
<td>Prophylactic is absent Treatment is imunomodulations</td>
</tr>
<tr>
<td><strong>Taxonomic position of virus of epidemic parotitis</strong></td>
<td>Family Paramyxoviridae Genus Rubulavirus RNA-virus</td>
</tr>
<tr>
<td><strong>Epidemic parotitis</strong></td>
<td>Sharp child’s infection which is characterized the defeat of parotid saliva glands, rarer – other organs</td>
</tr>
<tr>
<td><strong>Epidemiology of epidemic parotitis</strong></td>
<td>Sours of infections is sick human Way of transmission of aeroborn, rarely contact, through the objects infected saliva</td>
</tr>
<tr>
<td><strong>Basic stages of pathogenesis of epidemic parotitis</strong></td>
<td>Penetration of virus through entrance gates of the infection – overhead respiratory tracts</td>
</tr>
<tr>
<td></td>
<td>Reproduction of viruses is in the superficial epithelium overhead respiratory tracts, and possibly in parotid saliva glands</td>
</tr>
<tr>
<td></td>
<td>Virusemia, setting about a virus on an organism</td>
</tr>
<tr>
<td></td>
<td>Hit of virus in testis, pancreas and thyroid glands, brain-tunics, that results in their inflammation</td>
</tr>
<tr>
<td></td>
<td>As complications may be orchitis Asymptomatic</td>
</tr>
</tbody>
</table>
(and as a result – sterility), meningitis, meningocencephalitis, pancreatitis

| Methods of laboratory diagnostics of epidemic parotitis | 1. Virological method of diagnostics  
2. Serological method of diagnostics (ELISA, CFT, HAIT) |
|--------------------------------------------------------|--------------------------------------------------|
| Preparations for treatment and prophylaxis of epidemic parotitis | Specific immunoglobulin is for treatment and late prophylaxis.  
A specific prophylaxis is living vaccine. |
| Taxonomy of respiratory syncytial virus (RS-virus) | Family Paramyxoviridae  
Genus Pneumovirus  
RNA-virus |
| What is RS-infection? | Sharp disease of lower respiratory tracts in new-born and children of ranego age |
| Basic properties of the RS-virus | 1. It agglutinate erythrocytes  
2. A virus has characteristic morphology which appears at an electronic microscopy |
| Epidemiology of RS-infection | Sours of infections are sick human and viruscarrie.  
Way of transmission is aeroborn. |
| Features of pathogenesis of RS-infection | Entrance gate of of infection is overhead respiratory tracts  
Penetration of virus is in the epitalial cells  
Reproduction of virus in the cylinder epithelium cells of overhead respiratory tracts, which results in their death  
Generalizated of process with distribution on lower respiratory tracts  
Development of the second immunodeficient that results in development of the second bacterial infections  
Development of imunopatologic reactions as a result of formation of immune complexes |
| Methods of laboratory diagnostics of RS-infection | 1. Express-method of diagnostic (ELISA, IFT, rarer CFT, NT)  
2. Microscopic (histological) examination  
3. Virological method of diagnostics  
4. Serological method of diagnostics (NT, CFT, ELISA) |
| Taxonomy of the measles agent | Family Paramyxoviridae  
Genus Morbillivirus  
RNA-virus |
| What is measles? | Infectious disease, which is characterized a fever, catarrhal inflammation of shells of mucuses of overhead respiratory tracts and eyes, also spotty-papulosis pouring out on a skin. |
| Resistance of virus of measles is in an external environment | Quickly perish under act of different factors: temperature, acids, alkali, UFV and in, sensible to of tables disinfections. |
| Epidemiology of measles | A source of infection is a sick man. The people of different age are ill, but more frequent to put 4 – 5 years.  
Ways of transmission: basic - aeroborn, rarer - contact. Most infected in a prodromal period and in 1-st day of appearance of rash. In 5 days after appearance of rash there is a patient of not contagiosis. |
| Features of pathogenesis of measles | The pathogenesis of measles has not been fully explained. It is assumed that the virus, following primary replication in lymphoid tissues,
Measles is distributed hematogenously in two episodes. Thereafter the oral mucosa displays an enanthem and the tiny white “Koplik’s spots.” Then the fever once again rises and the typical measles exanthem manifests. Possible complications include otitis in the form of a bacterial superinfection as well as pneumonia and encephalitis. A rare late sequel of measles (one case per million inhabitants) is subacute sclerosing panencephalitis (SSPE) in which nucleocapsids accumulate in brain cells, whereby few or no viral progeny are produced for lack of matrix protein. This disease occurs between the ages of one and 20, involves loss of memory and personality changes, and usually results in death within six to 12 months.

Methods of laboratory diagnostics of measles
1. Express-method of diagnosis (IFT, PCR, ELISA, HAIT)
2. Virological method of diagnostics
3. Serological method of diagnostics (CFT, HAIT, NT, ELISA)

Specific prophylaxis of measles
The planned specific prophylaxis of measles is conducted subcutaneous introduction the children of the first year of life of living measles vaccine from attenuated cultures or associated vaccine (against measles, parotitis, German measles). The loosened children a normal human interferon is entered.

Taxonomic position of Coronaviruses infection agent
- Family Coronaviridae
- Genus Coronavirus
- RNA-virus

Resistance of coronaviruses
Viruses are sensible to acids, UFV; at heating to 56 C perish in 10-15 minutes. At a room temperature saved a few days. Bars to the low temperatures.

Epidemiology of coronaviruses
Sources of infections is sick human.
Way of transmission is aeroborn.

Features of pathogenesis of coronaviruses infection
Common coronaviruses cause an everyday variety of respiratory infections, which are restricted to the ciliated epithelia of the nose and trachea. They are responsible for about 30% of common cold infections.

The immunity conferred by infection, apparently IgA-dependent, is shortlived. Reinfections are therefore frequent, whereby the antigenic variability of the virus may be a contributing factor. Various enteral coronaviruses with morphologies similar to the respiratory types have also been described in humans. Their pathogenicity, and hence their contribution to diarrhea, has not been clarified.

The SARS virus is transmitted aerogenically with an incubation time of two to 10 days. Clinically, fever and a marked shortness of breath is noted, developing into a severe atypical pneumonia with new pulmonary infiltrates on chest radiography. Shedding of virus is by respiratory discharges. Whether the virus present in other body fluids and excreta plays a decisive role for virus transmission is not yet clear.

Methods of laboratory diagnostics of coronaviruses infection
1. Express-method of diagnosis (IFT)
2. Serological method (HAIT, CFT, NT).

Taxonomic position of adenoviruses infection agent
- Family Adenoviridae
- Genus Mastadenivirus
- DNA-virus

Epidemiology of adenoviruses infection
Sources of infections is sick human.
Way of transmission is aeroborn, “intestinal” adenovirus has food transmission way.

Pathogenesis of adenoviruses
Adenoviruses cause a variety of diseases, which may occur singly or
infections concurrently. The most important are infections of the upper (sometimes lower) respiratory tracts, the eyes, and the intestinal tract.

Infections of the respiratory tract take the form of rhinitis or abacterial pharyngitis, depending on the virus type as well as presumably on the disposition of the patient. They may also develop into acute, influenzalike infections or even, especially in small children, into a potentially fatal pneumonia.

The eye infections, which may occur alone but are often concurrent with pharyngitis, range from follicular conjunctivitis to a form of keratoconjunctivitis that may even cause permanent partial loss of eyesight.

### Diagnosis of adenoviruses infection

Antibody assays in patient serum are the main approach taken in respiratory adenovirus infections. Serology is unreliable in the eye and intestinal infections, since hardly any antibodies are produced in response to such highly localized infections. It is possible to isolate the viruses that cause respiratory infections by inoculating cell cultures with pharyngeal material or bronchial secretion and with conjunctival smears in eye infections. Enteral adenoviruses, on the other hand, are hard to culture. The best approach to detecting them is therefore to subject stool specimens to electron microscopy, enzyme immunoassay, or passive agglutination methods.

**Addition 2**

![Diagram of influenza nomenclature.](attachment:image)
Laboratory diagnostic of influenza

Express-method of diagnostic

- **Mucus from nasopharynx**, IFT - study virus’s antigens in material from patient, which luminescent specific antyinfluenzes serum
- **Saliva**, **PCR**
- **ELISA**, study sIgA

Virusological method

- **Washout from nasopharynx, excretion from nose, humidity**
- **I stage. Cultivation**
  - Infected chicken embryo in allantoises cavity
- **II stage. Indication**
  - HAT
  - III stage. Identification
    - HAIT, typing of viruses
- **I stage. Cultivation**
  - **Cell culture**
  - **II stage. Indication**
    - CPE
  - **III stage. Identification**
    - NT, and HAIT, Hemabsorbton inhibition test

Serological method

- **Pair serum of blood from patient**
  - “+” - if in reaction which pair serum from patient titer antibody increase in four times
  - **HAIT, CFT, PHAT**
"Laboratory diagnostic of parainfluenza"

Express-method of diagnostic
- In this method study the viruses in material from patient
  - Stroke (smear) from back wall of gullet and lower part nose, washout from nasopharynx
    - IFT
    - PCR

Virusological method
- Pus from back wall of gullet and lower part nose, autopsy material
  - I stage. Cultivation
  - Primary cell culture from kidneys of monkey, guinea pig, human’s embryo
    - II stage. Indication
    - After 2-4 days CPE or Hemabsorption test
      - III stage. Identification
      - Typing of viruses in NT or Hemabsorption inhibition test

Serological method
- Pair serum of blood from patient
  - CFT
    - NT
    - HAIT
Skim „Laboratory diagnostic of infectious parotitis”

**Express-method of diagnostic**
- In this method study the viruses in material from patient
  - Saliva, urine, cerebrospinal fluid
    - IFT
    - PCR

**Virusological method**
- Saliva, urine, cerebrospinal fluid
  - I stage. Cultivation
    - Infected chicken embryo in amnion cavity
    - Primary cell culture from kidneys of monkey, guinea pig, human’s embryo, cell culture Vero, BHK-21
  - II stage. Indication
    - HAT
    - CPE or Hemabsorption test
  - III stage. Identification
    - HAIT
    - NT or Hemabsorption inhibition test

**Serological method**
- Pair serum of blood from patient
  - CFT
    - ELISA
    - PHAT
Laboratory diagnostic is taking seldom. Often the diagnostic of the measles base on clinical symptoms.

**Express-method of diagnostic**
- In this method study viruses in material from patient
  - Mucus from nasopharynx, sediment of urine, autopsy
  - IFT
  - PCR

**Virusological method**
- Washout from nasopharynx, sediment from urine, mucus from conjunctive, autopsy
  - I stage. Cultivation
  - Primary cell culture from kidneys of monkey, guinea pig, human's embryo, cell culture Hela, Hep-2
  - II stage. Indication
  - CPE (to formation of the syncitium) or Hemabsorption test
  - III stage. Identification
  - Typing of viruses in NT or Hemabsorption inhibition test

**Serological method**
- Pair serum of blood from patient
  - CFT
  - PHAT
  - NT
  - HAIT
Skim „Laboratory diagnostic of RS-infection”

**Express-method of diagnostic**
- In this method study viruses in material from patient
  - Washout from nasopharynx or oral cavity
    - IFT
    - RID
    - ELISA

**Virusological method**
- Pus from nasopharynx, nose, oral cavity, autopsy
  - I stage. Cultivation
    - Cell culture Hela, Hep-2, Vero
      - II stage. Indication
      - CPE or Hemabsorption test
        - III stage. Identification
        - Typing of viruses in NT or Hemabsorption inhibition test

**Serological method**
- Pair serum of blood from patient
  - CFT
    - ELISA
      - NT
      - HAIT

**Microscopic (histology) examination**
- In epithelial cells of mucosa membrane of bronchi exposure polinuclear cells and sincity
Skim „Laboratory diagnostic of respiratory adenoviruses infection”

Express-method of diagnostic

In this method study viruses in material from patient

Mucus from nasopharynx, conjunctive

IFT

PCR

EM

ELISA

Virusological method

Mucus from nasopharynx, conjunctive

I stage. Cultivation

Cell culture Hela, Hep-2, Vero

II stage. Indication

After 2 – 4 days CPE

III stage. Identification

Typing of viruses in NT

Serological method

Pair serum of blood from patient

CFT

ELISA

NT

HAIT