LABORATORY DIAGNOSTICS OF COVID INFECTION

Methodical instruction

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

LABORATORY DIAGNOSTICS OF COVID INFECTION

Methodical instruction
for students II–III courses for medical and dental faculties
from the discipline «Microbiology, virology and immunology»

ЛАБОРАТОРНА ДІАГНОСТИКА КОВІДНОЇ ІНФЕКЦІЇ

Методичні вказівки
для студентів II–III курсів
медичного та стоматологічного факультетів
з дисципліни «Мікробіологія, вірусологія та імунологія»

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Compilers: M. M. Mishina
O. V. Kotsar
O. V. Kochnieva
M. M. Kovalev
U. M. Kalashnik-Vaculenko

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Упорядники: М. М. Мішина
О. В. Коцар
О. В. Кочнєва
М. М. Ковальов
Ю. М. Калашник-Вакуленко

INTRODUCTION

Coronavirus infection (COVID-19) is an acute infectious disease caused by a new strain of the virus of the SARS CoV-2 family of coronaviruses

The pandemic has resulted in the collapse of civilization and death of most of earth's population and the travel restrictions and nationwide lockdowns in several countries.

The rapid spread of the disease, high mortality rate and the development of severe complications have posed challenge for specialists related to rapid diagnosis and provision of medical care to the patients.

The most common clinical manifestation of the new variant of coronavirus infection is bilateral pneumonia (viral diffuse alveolar damage with microangiopathy), acute respiratory failure (ARF), and the development of acute respiratory distress syndrome (ARDS) has been reported in 3-4% of patients. Some patients develop hypercoagulation syndrome with thrombosis and thromboembolism; the other organs and systems are also affected (central nervous system, myocardium, kidneys, liver, gastrointestinal tract, endocrine and immune systems), and the sepsis and septic shock occurs.

Researchers from all countries continue to study the clinical and epidemiological features of the disease, the developing new means of disease prevention, control and treatment in order to stop the further spread of the COVID infection.

The purpose of the study:

- **General:** to master the principles of laboratory diagnosis of COVID-19
- **Specific:**

a) to know:

- biological properties, epidemiology, pathogenesis, clinical manifestations, methods of treatment and prevention of COVID-19.

b) to be able to:

- interpret the results of laboratory tests for COVID-19.

Material and methodological support for the topic: visual aids (tables, diagrams, slides, atlas); laboratory equipment (microscopes, immersion oil, disinfectant solution); technical teaching aids (computer, interactive whiteboard).

Taxonomy

Coronaviruses (*Coronaviridae*) is a large family of RNA viruses that can infect both animals (their natural hosts) and humans.

Based on the results of serological and phylogenetic analysis, coronaviruses are divided into two subfamilies Letovirinae and Orthocoronavirinae, which includes four genera:

- Alphacoronavirus,
- Betacoronavirus,
- Gammacoronavirus,
- Deltacoronavirus.

History of the discovery of coronaviruses

The virus was first isolated in 1965 by Joseph Tyrrell from the nasopharynx of a patient with acute rhinitis. In 1975, coronavirus was discovered by E. Kaul and

S. Clark in the feces of children suffering from gastroenteritis. Until 2002 coronaviruses did not attract much attention from scientists.

Currently, four coronaviruses are known to circulate among the population (HCoV 229E, OC43, NL63 and HKU1), which are present year-round in the structure of acute respiratory infections. As a rule, it causes mild to moderate damage to the upper respiratory tract.

- HCoV-229E – Alphacoronavirus, first identified in the mid-1960s;
- HCoV-OC43 – Betacoronavirus, the pathogen was identified in 1967;
- SARS-CoV – Betacoronavirus, the causative agent of severe acute respiratory syndrome, the first case of which was registered in 2002;
- HCoV-NL63 – Alphacoronavirus, the pathogen was identified in the Netherlands in 2004;
- HCoV-HKU1 – Betacoronavirus, the pathogen was discovered in Hong Kong in 2005;
- MERS-CoV – Betacoronavirus, the causative agent of Middle East respiratory syndrome, an outbreak occurred in 2015;

SARS-CoV-2 – Betacoronavirus, identified in the second half of 2019.

In the period from 2002 to 2004 Coronavirus SARS-CoV from the genus Betacoronavirus is the first caused the development of an epidemic, the so-called atypical pneumonia – severe acute respiratory syndrome (SARS or SARS). The disease has become a cause of death for 774 people in 37 countries. It is known that the reservoir of this infection were bats. Since 2004, no new cases of atypical pneumonia caused by SARS-CoV have been reported.

In 2012, another epidemic began on the Arabian Peninsula, caused by the MERS-CoV coronavirus, also from the genus Betacoronavirus, which caused the Middle East coronavirus syndrome and 82 % of cases were detected in Saudi Arabia. It was found that the reservoir of infection was dromedary camels. Currently, MERS-CoV continues to circulate and causes the new cases of the disease.

At the end of 2019, an outbreak of a new coronavirus infection occurred in the People's Republic of China with the epicenter in the city of Wuhan (Hubei Province). The official date of occurrence of the disease is November 17, 2019. On February 11, 2020, the World Health Organization (WHO) determined the official name of the infection caused by the new coronavirus – COVID-19 (“Coronavirus disease 2019”). On February 11, 2020, the International Committee on Taxonomy of Viruses assigned the official name to the infectious agent – SARS-CoV-2. Over time, the virus spread to all continents, causing the WHO to declare a pandemic on March 11, 2020.

Morphological structure of the SARS-CoV-2 virus

SARS-CoV-2 is an enveloped RNA virus with a helical symmetry. The virion diameter is 50–200 nm. SARS-CoV-2 virion has a spherical shape, but pleomorphic and oval shapes are also found. There are three proteins on the surface of the supercapsid: protein S – spike glycoprotein, which forms peplomeres and gives the virus a characteristic crown shape, M membrane glycoprotein and E – envelope protein. The fourth protein is N-nucleocapsid, phosphoprotein that is a structural component of the nucleocapsid (Fig. 1).

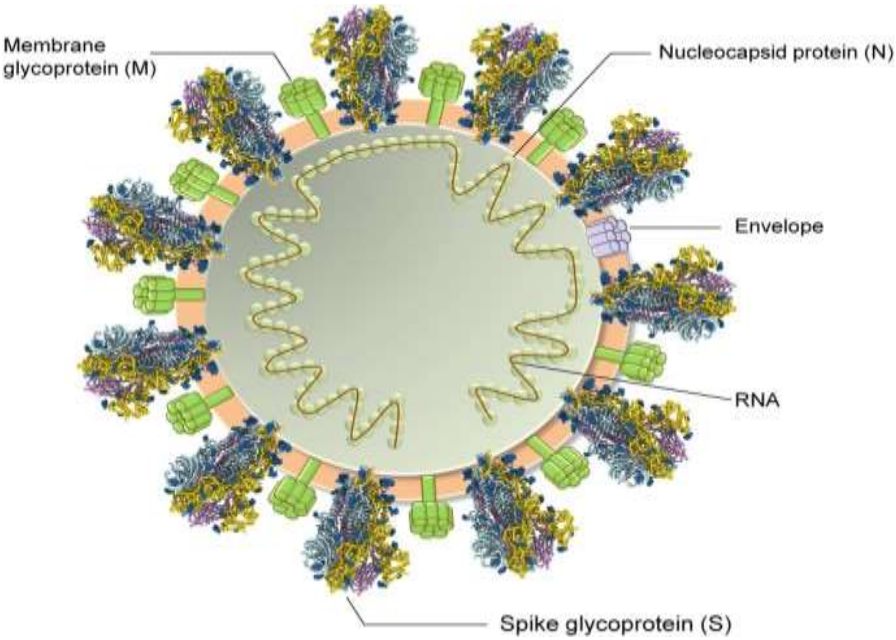


Fig. 1. Structure of SARS-CoV-2

Protein S is a glycoprotein characterized by a homotrimeric structure with one upper and two lower conformations. The identity amino acid sequence of the S protein between SARS-CoV-1 and SARS-CoV-2 is about 75.5 %. Protein S consists of two subunits (N'-terminal S1 and C'-terminal S2) which are involved in binding to host cell receptors and endocytosis of the virion. There are 4-amino acid regions which take part in the breakdown of furin protein during biosynthesis. It distinguishes SARS-CoV-2 from SARS-CoV-1. The S1 and S2 subunits of SARS-CoV-2 have approximately 64 % and 90 % similarity with the corresponding subunits in SARS-CoV-1.

Structural protein E is involved in the formation of new virions which are mainly constructed from host cell material. It contains a large amount of valine and contains less guanine and cytosine than the similar structural protein of SARS-CoV-1.

The genome of the virus consists of single-stranded, linear, positively charged, non-fragmented RNA, ranging from 26 to 32 thousand nucleotide pairs. Coronaviruses have the largest genome of any RNA virus family.

Replication of coronavirus

- penetration of the virus into the cell using the S protein (receptor for 2019-nCoV – angiotensin-binding protein);
- translation of polyproteins and processing of the replication complex;
- replication and transcription of the virus;
- synthesis of structural proteins;

- assembly and budding of viral particles from the ER and Golgi complex;
- virus release via exocytosis.

Coronaviruses adsorb onto the cell using glycoprotein S, enter the cell when the viral envelope fusions with the cell's cytoplasm membrane or through receptor endocytosis (fig. 2). Genomic RNA binds to ribosomes and serves as iRNA in the synthesis of RNA-dependent RNA polymerase, which then reads the genomic RNA, synthesizing the full-length minus strand. When reading minus-thread RNA, a new genomic plus-strand RNA and of 5–7 subgenomic iRNAs are synthesized. One protein is produced by translation each subgenomic iRNA. Protein N binds to genomic RNA in the cytoplasm, resulting in the synthesis of a helical nucleocapsid.

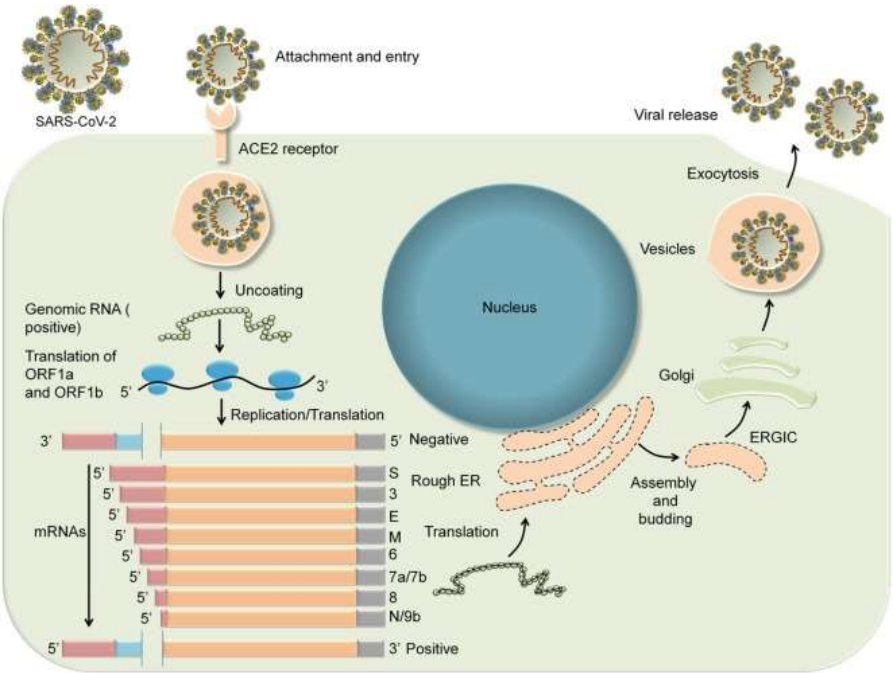


Fig. 2. Replication cycle of SARS-CoV-2

Glycoproteins S and M, or E1, E2 are transported to the endoplasmic reticulum (ER) and Golgi apparatus (AG). The nucleocapsid buds through membranes into the rough endoplasmic reticulum containing glycoproteins S and M. Virions are transported in vesicles to the cell membrane. Virions leave the cell by exocytosis.

Cultivation. Viruses are cultivated in cell culture: continuous HeLa cells; primary trypsinized culture of pig embryonic kidney cells.

Resistance.

- Environmental stability is low
- Dies under the influence of ultraviolet radiation, disinfectants
- When heated to 40 °C, it dies within 1 hour
- When heated to 56 °C, it dies within 30 minutes.
- On the surface of objects at 18–25 °C it remains viable for 2 to 48 hours.

Pathogenesis. The entry gate of the pathogen is the epithelium of the upper respiratory tract and epithelial cells of the stomach and intestines. The initial stage of infection is the penetration of SARS-CoV-2 into target cells that have angiotensin-converting enzyme type II (ACE2) receptors. The main and quickly accessible target is alveolar type II cells (AT2) of the lungs, which determines the development of pneumonia. The role of CD147 in SARS-CoV-2 cell invasion is also discussed. Dissemination of SARS-CoV-2 from the systemic circulation or through the lamina cribrosa can lead to brain damage.

Hyposmia in a patient at an early stage of the disease may indicate damage to the central nervous system.

Diffuse alveolar damage develops. The virus causes an increase in the permeability of cell membranes and increased transport of albumin-rich fluid into the interstitial tissue of the lung and the lumen of the alveoli. Interstitial and alveolar edema develops. In this case, the surfactant is destroyed, which leads to the collapse of the alveoli, and as a result of a sharp disturbance in gas exchange, acute respiratory distress syndrome develops (fig. 3).

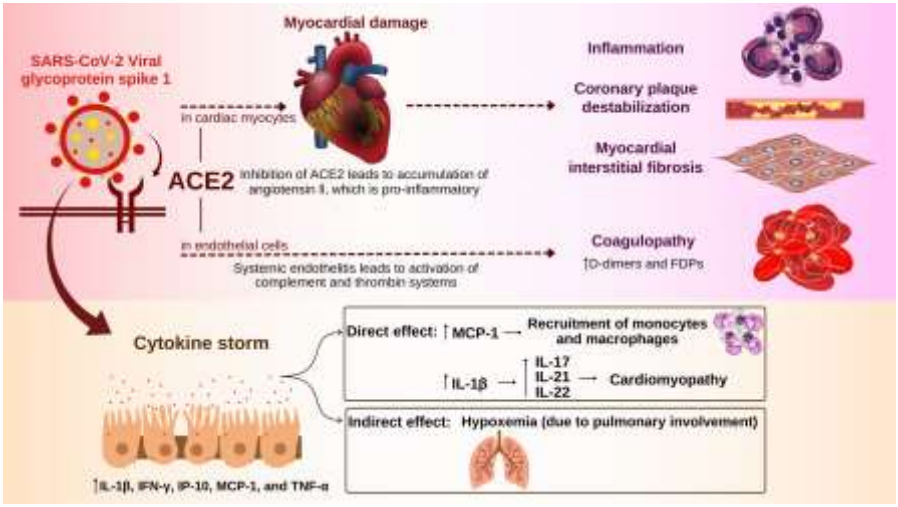


Fig. 3. Pathogenesis SARS-CoV-2

Exudative (acute) stage. Damage to type I alveocytes – increased permeability of the alveolo-capillary cell membrane – interstitial and alveolar edema – filling of the alveoli with leukocytes, erythrocytes, products of destroyed cells (flooding of the alveoli, disruption of function and production of endogenous surfactant).

Proliferative (subacute) stage. Damage to type II alveocytes>migration of fibroblasts into alveolar exudate>proliferation of type II alveocytes>reduction of pulmonary edema.

Fibroproliferative (chronic) stage. Obliteration of the alveoli leads to the severe fibrosis of the pulmonary parenchyma.

Epidemiology

Natural reservoir – unknown (probably wild animals).

SARS-CoV-2 is a recombinant virus between a bat coronavirus and a coronavirus of unknown origin.

Source of infection: sick person (during the incubation period and at the height of the disease); asymptomatic carriage with the possibility of transmission from a clinically healthy person to contact persons is not excluded.

The incubation period lasts from 2 to 14 days, with an average of 5–7 days.

The new form of COVID-19 is characterized by the presence of clinical symptoms of acute respiratory infection (ARI):

- temperature of body increases (>90 %);
- cough (dry or with scant sputum) in 80 % of cases;
- shortness of breath (55 %);
- myalgia and fatigue (44 %);
- feeling of chest congestion (>20 %).

There is also a sore throat, runny nose, decreased sense of smell and taste, and signs of conjunctivitis. The most severe shortness of breath develops by 6–8 days from the moment of infection. The first symptoms may be myalgia (11 %), confusion (9 %), headaches (8 %), hemoptysis (5 %), diarrhea (3 %), nausea, vomiting, palpitations. These symptoms of infection can be observed without of increasing body temperature (fig. 4.).

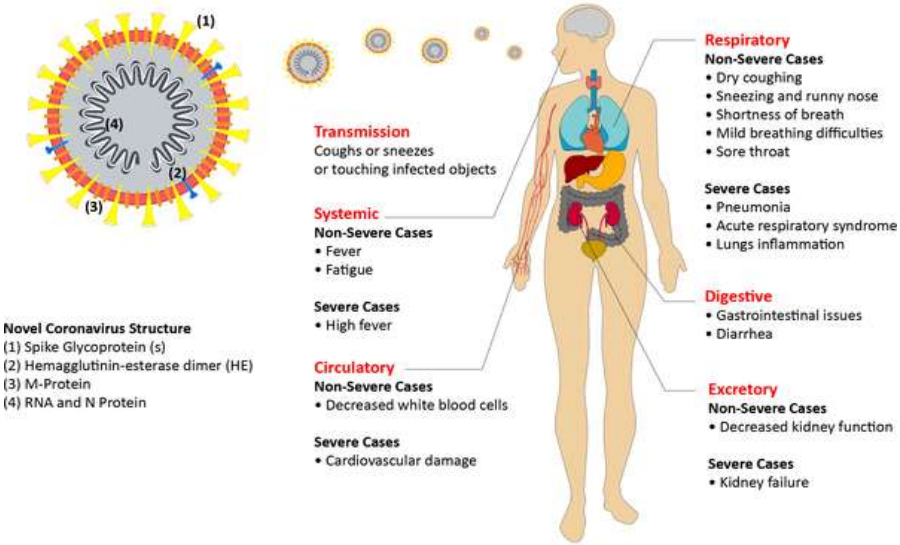


Fig. 4. Clinical symptoms of SARS-CoV-2

Clinical variants and manifestations of COVID-19:

1. Acute mild respiratory viral infection.
2. Pneumonia without acute respiratory failure (ARF).
3. Pneumonia with respiratory failure.
4. Respiratory failure.
5. Sepsis.
6. Septic (infectious-toxic) shock.

Hypoxemia (decrease in SpO2 less than 88 %) develops in more than 30 % of patients. Lung damage of more than 50 % is considered dangerous and requires hospitalization of the patient, especially if the pulse oximeter shows saturation (oxygen saturation of the blood) less than 92–93 %.

There are mild, moderate and severe forms of COVID-19 infection.

Risk factors for developing severe forms of COVID-19:

- obesity;
- elderly age;
- chronic bronchopulmonary diseases;
- diabetes;
- arterial hypertension;
- oncological diseases.

Severe forms of COVID-19 infection account for 25 % of confirmed cases of the disease, of which 16 % are seriously ill, 5 % are in critical condition and 4 % are dead.

In severe cases, rapidly progressive damage to the lower respiratory tract, pneumonia, ARF, sepsis and septic shock are observed. In Wuhan, almost all patients with severe disease developed progressive ARF: pneumonia was diagnosed in 100 % of patients, and ARDS in more than 90 % of patients.

On average, 50 % of infected people are asymptomatic. In 80 % of patients with clinical symptoms, the disease occurs in a mild form of ARVI.

Cytokine storm is an important factor influencing the outcome of COVID-19.

Cytokine storm syndrome is an immunopathological condition characterized by a sharp increase in proinflammatory cytokines after stimulation of the body by microorganisms or drugs. Under normal conditions, levels of pro-inflammatory cytokines and anti-inflammatory cytokines in the body remain relatively balanced. When the virus enters the body, the immune system is over-activated, including dendritic cells, macrophages, lymphocytes and natural killer cells. These cells secrete a large number of cytokines: IL1, IL2, IL6, IL7, IL8, IL9, IL10, IL12, IL17, IL18, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor a (TNFa), IFN?-inducible protein 10, IFNa and IFNß, monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1a (MVB1a), and inflammatory markers such as C-reactive protein and ferritin increase.

Clinical recovery of patients with COVID-19

A patient can be considered officially recovered if:

- 10 days have passed since the first day of symptoms and there have been no symptoms for 3 days;

- 10 days have passed since receiving a positive PCR test result, there were no symptoms for 3 days;
- a negative PCR test result was received.

Post-COVID syndrome (Post-COVID long-haule) occurs in patients who were diagnosed with COVID-19 and who have not returned to full recovery 6 months after the illness. Such manifestations are observed in 10 to 50 % of cases. People with post-Covid syndrome are divided into 2 groups. 1. persons who have permanent damage to the lungs, heart, kidneys or brain that affects their ability to function; 2. Individuals who have debilitating symptoms despite no noticeable organ damage. The presence of such clinical manifestations or the appearance of new symptoms after an acute illness necessitates the need for rehabilitation of such patients.

Manifestations of post-Covid syndrome are very different and are observed for several months after the infection. The main symptom is increased fatigue (in 100 % of cases). After recovery, 90 % of patients experience intense headaches, which may be accompanied by vestibular disorders: hearing and vision impairment. Ophthalmic changes following COVID-19 are associated with eye diseases such as conjunctivitis. 60 % of patients have mental disorders, 20 % have depression, bad mood, tearfulness, 28 % have anxiety symptoms, and 28–56 % have various problems with memory and intelligence. Over the long term, 75 % of patients may experience hypothermia or hyperthermia. Sore throat occurs in 85 %, soreness of the lymph nodes – 80 %, myalgia 80 %, joint pain 80 %, paresthesia, impaired movement and sensitivity in various parts of the body. There may also be problems with hearing, hair falling out, and teeth deteriorating. Some men experience impotence.

Clinical manifestations of COVID-19 in elderly patients

Elderly patients may have an atypical picture of illness without fever and cough due to reduced body reactivity. Symptoms of COVID-19 may be subtle and inconsistent with the severity of the disease and prognosis. Possible atypical symptoms of COVID-19 in elderly and senile patients: delirium and delirium.

Clinical manifestations in children of COVID-19

Children get sick less often with COVID-19, the clinical picture is less pronounced, they require hospitalization less often, and their disease is milder. However, severe cases cannot be excluded.

Available data indicate that children make up up to 10% of those infected with SARS-CoV-2 and up to 2 % of patients with diagnosed clinical cases of COVID-19.

Risk factors differ significantly in children, because. only in 9 % of cases, infection with the SARS-CoV-2 virus is associated with travel to other countries and 91 % of cases had local contacts, mainly in family members.

Children have a milder course of the disease compared to adults, viral pneumonia is not typical, symptoms are less pronounced, and deaths are extremely rare. In children, as in adults, fever and respiratory syndrome dominate, but lymphopenia and inflammatory markers are less pronounced.

The incubation period in children ranges from 2 to 10 days, with an average of 2 days.

Clinical symptoms of COVID-19 in children are similar to the clinical picture of ARVI: fever, cough, sore throat, sneezing, weakness, myalgia. The severity of the febrile reaction can be different: a temperature of 38 °C is observed in half of the sick children, in a third of children the temperature ranges from 38.1 to 39.0 °C. The accumulated experience of monitoring the clinical manifestations of COVID-19 in children has shown that the combination of fever, cough and shortness of breath occurs only in 73 %, while in adults – 93 %. According to US researchers, only 56 % of pediatric patients reported fever, 54 % cough and 13 % shortness of breath, compared with 71 %, 80 % and 43 %, respectively, among adult patients aged 18–64 years. Children are less likely to experience clinical manifestations of intoxication (headache in 28 %, in adults 58 %; myalgia – 23 %, 61 % of adults). Symptoms of respiratory failure and gastrointestinal manifestations (nausea/vomiting) are much less common in children with COVID infection.

According to the data, from 5,7 % to 20 % of children with COVID-19 were treated in the hospital, the rest were treated on an ambulatory, like patients with mild, subclinical and asymptomatic forms in whom the SARS-CoV-2 virus was isolated during contact. In the United States, only 1,6–2,5 % of children with COVID-19 required hospitalization, and there was no need for reanimation help.

Clinical symptoms of coronavirus infection range from no symptoms (asymptomatic) or mild respiratory symptoms to a severe form occurring with:

- high fever;
- severe impairment of consciousness;
- chills, sweating;
- headaches and muscle pain;
- dry cough, shortness of breath, rapid and difficult breathing;
- increased heart rate.

The most common complication of acute respiratory syndrome caused by COVID infection is bilateral viral pneumonia, which leads to pulmonary edema. Respiratory arrest is possible, which requires emergency assistance and the use of artificial ventilation.

The following complications are also possible: acute respiratory distress syndrome; acute heart failure; abdominal pain; diarrhea, development of acute renal failure; septic shock; multiple organ failure (impaired functions of many organs and systems).

It is noted that not all children with suspected COVID-19 with a severe form of the disease had laboratory confirmed SARS-CoV-2 virus. This can be explained by co-infection or the presence of other respiratory diseases in children with suspected cases of disease.

Mild severity is characterized by an increase in body temperature no higher than 38.5 °C, the absence of shortness of breath at rest, but it may appear during physical activity, $SpO2 > 95$ %.

Moderate severity is characterized by raise the body temperature above 38.5 °C, the absence of shortness of breath at rest, but its appearance during physical activity (screaming/crying), $SpO2 \leq 95$ %.

Severe COVID-19 is characterized by dyspnea (shortness of breath, chest tightness, shortness of breath or tachypnea), cyanosis/acrocyanosis, SpO2 ≤ 93 %.

An extremely severe degree is recorded with the development of respiratory failure with the need for respiratory support, acute respiratory distress syndrome, shock, signs of multiple organ failure (encephalopathy, cardiovascular, renal, liver failure, disseminated intravascular blood clotting syndrome).

The frequency of severe and extremely severe cases of the disease does not exceed 1 %. Extremely severe forms of COVID-19 usually occur in children in the presence of risk factors: severe premorbid diseases (children with lung diseases, congenital heart defects, bronchopulmonary dysplasia, Kawasaki disease, hydronephrosis, leukemia, etc.), immunodeficiency states of various origins (they get sick more often children over 5 years old; pneumonia is 1.5 times more likely to be recorded). It is worth paying attention to the effect of co-infection with other respiratory viruses (respiratory syncytial virus, rhinovirus, bocavirus, adenovirus), which are characterized by damage to the lower respiratory tract (pneumonia, bronchiolitis).

For asymptomatic or mild COVID-19, treatment is carried out on an outpatient basis, including at home.

Features of pregnancy with COVID infection

Obstetric tactic depends on the following factors: the severity of the patient's condition, the condition of the fetus and gestational age. Moderate and severe degrees of disease before the 12^{th} week of gestation can lead to dangerous complications that are caused by the effects of a viral infection and the embryotoxic effect of drugs. Possible termination of pregnancy is noted after the infectious process is cured. If the patient refuses to terminate the pregnancy, then it is necessary to perform a chorionic villus biopsy or placenta before 12–14 weeks or amniocentesis from 16 weeks of gestation to identify chromosomal abnormalities of the fetus.

Termination of pregnancy and delivery at the height of the disease is associated with an increase in complications: the development and progression of respiratory failure, the occurrence of obstetric hemorrhages, intrapartum fetal death and postpartum purulent-septic complications.

However, if it is impossible to eliminate hypoxia or with the progression of respiratory failure, the development of alveolar pulmonary edema, as well as with septic shock, in the interests of the mother and fetus, emergency abdominal delivery (cesarean section) is recommended with all necessary measures to prevent coagulopathic and hypotonic obstetric hemorrhage. In pregnancy up to 20 weeks, an emergency caesarean section may not be performed, since the pregnant uterus at this stage does not affect cardiac output. At 20–23 weeks of pregnancy, an emergency caesarean section is recommended to save the life of the mother, but not the fetus, and at more than 24 weeks - to save the life of the mother and fetus.

In the case of spontaneous labor in the midst of a disease (pneumonia), it is preferable to give birth through the natural birth canal under the control of the condition of the mother and fetus.

To prevent the development of respiratory and cardiovascular failure, efforts should be weakened in the second stage of labor. In order to speed up the process of

delivery in case of fetal distress, weakness of labor and/or deterioration of the woman's condition, it is possible to use vacuum extraction or obstetric forceps.

The prognosis for the mother and fetus depends on the trimester of gestation in which the disease occurred, the presence of premorbid background (smoking, obesity, underlying diseases of the respiratory system and ENT organs, diabetes mellitus, HIV infection), the severity of the infectious process, the presence of complications and timeliness initiation of antiviral therapy.

Laboratory diagnosis of COVID infection

General laboratory diagnostics.

General (clinical) blood test. It includes determination of the level of red blood cells, hematocrit, leukocytes, platelets, and leukocyte formula. The neutrophil/lymphocyte ratio is one of the leading prognostic factors. Patients with COVID-19 have lymphopenia.

Biochemical blood test is determination of the level of urea, creatinine, electrolytes, liver enzymes, bilirubin, glucose, albumin. This diagnostic method does not provide any specific information, but makes it possible to identify abnormalities that may indicate the presence of organ dysfunction, decompensation of concomitant diseases and the development of complications, have a certain prognostic value, and influence the choice of medications and/or their dosage regimen.

Determination of the level of C-reactive protein (CRP) in blood serum.

The level of CRP correlates with the severity of the disease, the prevalence of inflammatory infiltration and the prognosis of pneumonia. CRP is the main laboratory marker of process activity in the lungs. Its increase is the basis for starting anti-inflammatory therapy.

Hormonal tests - determination of procalcitonin, brain natriuretic peptide - NT-proBNP/BNP. Procalcitonin in coronavirus infection with damage to the respiratory parts of the lungs is within the reference values. An increase in procalcitonin indicates the addition of a bacterial infection and correlates with the severity of the course, indicating the spread of inflammatory infiltration.

Coagulograma is determination of prothrombin time, prothrombin index, fibrinogen, D-dimer (quantitative method). There is an increase in D-dimer 3–4 times higher than age norm and prolongation of prothrombin time, especially in severe cases (decrease in % prothrombin), an increase in fibrinogen is of great clinical importance.

Specific laboratory diagnostics

Direct methods of etiological diagnosis.

- Detection of SARS-CoV-2 RNA by PCR is of primary importance for the etiological laboratory diagnosis of COVID-19.
- Detection of SARS-CoV-2 antigen using immunochromatographic methods.

Indirect methods of etiological diagnosis.

Detection of immunoglobulins of classes A, M, G (IgA, IgM and IgG) to SARS-CoV-2 (including the receptor-binding domain of surface glycoprotein S) by ELISA.

The main biomaterial for laboratory testing for SARS-CoV-2 RNA is material obtained by collecting a smear from the nasopharynx (from two nasal passages) and the oropharynx. Swabs from the mucous membrane of the

nasopharynx and oropharynx are collected in one tube to obtain a higher concentration of the virus.

If there are signs of damage to the lower respiratory tract, if a negative result is obtained in smears from the mucous membrane of the nasopharynx and oropharynx, sputum (if available) or bronchial lavage water obtained during fibrobronchoscopy (bronchoalveolar lavage), endotracheal, nasopharyngeal aspirate are additionally examined. In intubated patients (patients receiving mechanical ventilation), it is recommended to obtain and examine a tracheal aspirate to detect SARS-CoV-2.

Lung biopsy, whole blood, serum and feces can be used as ***additional research material***.

All samples received for laboratory testing should be considered potentially infected, and when working with them, the requirements "Safety of working with microorganisms of pathogenicity groups I – II" must be taken into account. Health care workers who collect or transport clinical specimens to the laboratory should be trained in safe handling practices, adhere to strict safety precautions, and use personal protective equipment.

Rules for collecting material from the nasopharynx for testing for SARS-CoV-2

The material is collected using a dry sterile swab. Nasopharyngeal and oropharyngeal swabs should be placed in one tube to increase the viral load + 2 ml phisiological solution.

1. The patient should sit with a straight back or with a headrest; the head can be placed vertically or slightly tilted back.

2. Inform the patient that the procedure may be uncomfortable because the swab probe must be inserted deeply.

3. Ask the patient to remove the mask (pull it off the nose) and clean the nose with a disposable tissue.

4. Measure the distance between the nostrils and the external auditory canal to estimate the distance to which the swab probe should be inserted (the actual depth to which the swab probe should be inserted is 1–2 cm). Before unpacking the swab probe, make sure that the specified distance corresponds to the distance between the tip of the swab probe and the manufacturer's mark (Fig. 5).

Detection of antibodies to SARS-CoV-2 is of auxiliary value for diagnosing a current infection and is fundamental for assessing the immune response to a current or previous infection. Antibodies to SARS-CoV-2 are detected using immunochemical methods. The decision to test for antibodies to SARS-CoV-2 is made individually by the attending physician, based on clinical appropriateness.

Antibodies of class (IgA) begin to form and are available for detection from approximately 2 days from the onset of the disease, reach a peak after 2 weeks and persist for a long time.

Antibodies of class M (IgM) begin to be detected approximately on the 7^{th} day from the onset of infection, reach a peak after a week and can persist for 2 months or more.

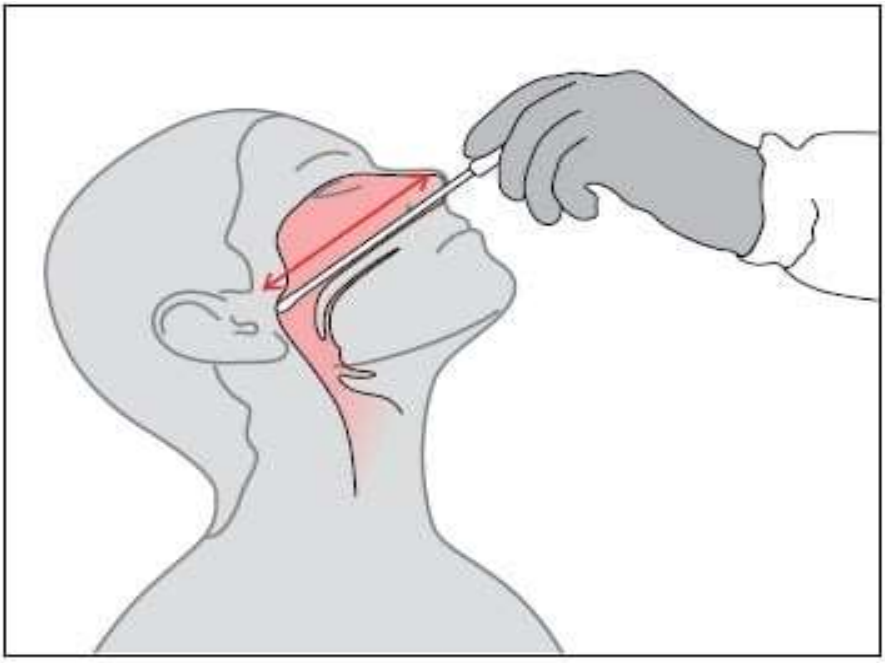


Fig. 5. Collecting material from the nasopharynx for testing for SARS-CoV-2

Antibodies of class G (IgG) appear from about the 3rd week or earlier to SARS-CoV-2. A feature of the humoral response to infection is the short time interval between the appearance of IgM and IgG antibodies, and sometimes their simultaneous formation.

To diagnose COVID-19 using the indirect method, it is recommended to conduct separate testing for IgM/IgA and IgG antibodies, as well as monitoring the appearance of antibodies over time. In unclear cases, retesting is recommended after 5–7 days.

To determine the presence of IgG, it is recommended to use reagents for quantitative determination of antibody titer, which will allow assessing the strength of immunity over time and will allow the selection of immune plasma for potential donors.

To minimize false results, it is recommended to introduce an algorithm for sequential examination of patients who have received initial positive results for antibodies of the IgM/IgA or IgG classes, an additional test should be used with maximum sensitivity and specificity, as well as with the simultaneous detection of antibodies of classes A, M, G. B As such a test system, a test system can be used to detect the receptor-binding domain of the surface glycoprotein S of SARS-CoV-2. The use of such an algorithm will allow for reliable diagnostics.

Blood testing for antibodies to the SARS-Cov-2 virus is recommended in the following cases:

- as an additional method for diagnosing acute infection

- if it is not possible to test smears by amplifying nucleic acids
- during hospitalization for treatment of somatic pathology;
- to identify individuals with asymptomatic infection;
- to establish a previous infection
- conducting a mass survey of the population to assess the level of population immunity;
- to select potential immune plasma donors.

Testing for total antibodies (IgA, IgM and IgG) or IgG to SARS-CoV-2 is recommended weekly for all health care workers who have not previously had such testing or if the result was negative. If IgG to SARS-CoV-2 appears as a result of previous infection or vaccination, further testing for antibodies to the virus is not carried out.

When assessing the intensity of post-vaccination protective immunity using enzyme immunoassay, it is advisable to determine antibodies to the receptor-binding domain (anti-RBD antibodies).

As a material for conducting laboratory tests for the presence of IgA, IgM and/or IgG (in individual studies or in total) against SARS-CoV-2, blood or other types of biomaterials are used in accordance with the instructions for the reagent kit used (*Fig. 6*).

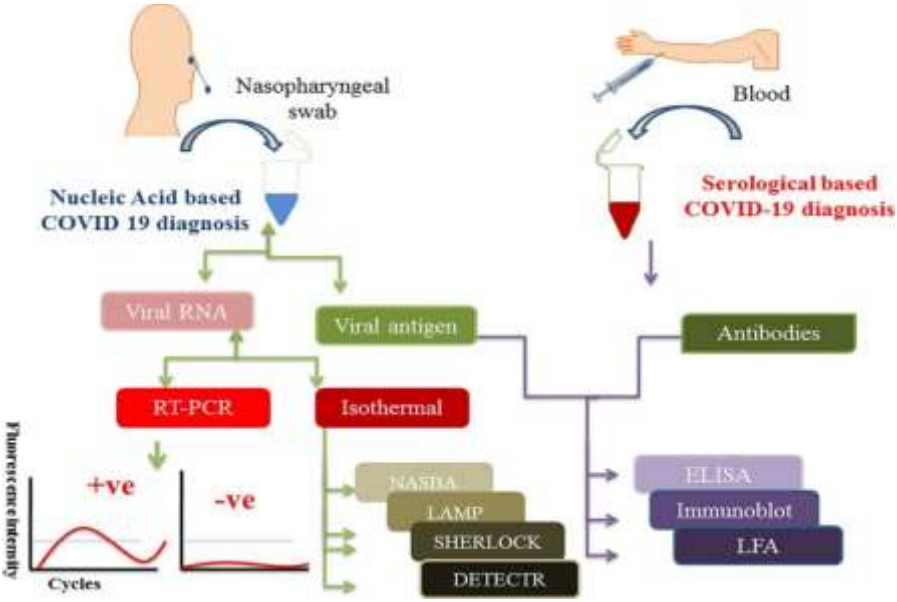


Fig. 6. Scheme of laboratory diagnosis of SARS-CoV-2

To carry out differential diagnosis, PCR studies are carried out in all patients using the PCR method for pathogens of respiratory infections: influenza viruses type A and B, respiratory syncytial virus (RSV), parainfluenza viruses, rhinoviruses, adenoviruses, human metapneumoviruses, MERS-CoV.

It is necessary to carry out microbiological diagnostics (culture examination) and/or PCR method for the presence of Streptococcus pneumoniae, Haemophilus influenzaetype B, Legionella pneumophila, as well as other pathogens of bacterial respiratory infections of the lower respiratory tract.

RADIATION DIAGNOSIS AT COVID-19

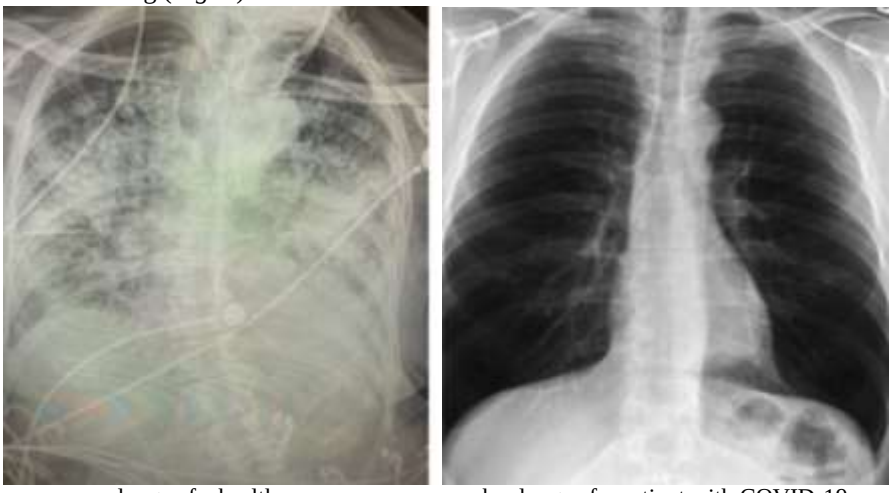
Radiation diagnostic methods are used to identify COVID pneumonia, complications, for differential diagnosis with other lung diseases, as well as to determine the extent, dynamics of the process and evaluate the effectiveness of therapy.

Radiation methods are necessary to identify and evaluate the nature of pathological changes in other anatomical areas, as a means of control for invasive medical interventions.

Methods for radiological diagnosis of pathology of the chest organs of patients with suspected/determined COVID-19 pneumonia include:

- Plain radiography of the lungs (RG),
- Computed tomography of the lungs (CT),
- Ultrasound examination of the lungs and pleural cavities (ultrasound).

Standard radiography has low sensitivity in detecting initial changes in the first days of the disease and is not used for early diagnosis. The information value of this study increases with increasing duration of pneumonia. The method makes it possible to detect severe forms of pneumonia and pulmonary edema of various natures, which require hospitalization. X-ray of the lungs allows you to determine the symptom of "white lungs" - the image looks almost completely white. This may indicate that the lungs are filled with fluid, bacteria, and have lesions and dense scarring (*Fig. 7*).



a – lungs of a healthy person b – lungs of a patient with COVID-19

Fig. 7. X-ray examination of the lungs

Computed tomography (CT) has high sensitivity in detecting changes in the lungs characteristic of COVID-19. The use of CT is advisable for the initial assessment of the condition of the chest organs in patients with severe progressive forms of the disease, as well as for the differential diagnosis of identified changes and assessment of the dynamics of the process. CT scans can detect characteristic changes in the lungs of patients with COVID-19 even before positive laboratory tests for infection appear. At the same time, CT detects changes in the lungs in a significant number of patients with asymptomatic and mild forms of the disease who do not require hospitalization. The results of CT in these cases do not affect the treatment tactics and prognosis of the disease in the presence of laboratory confirmation of COVID-19, therefore the widespread use of CT for screening asymptomatic and mild forms of the disease is not recommended.

The limitations of CT are due to the lower availability of technologies in individual medical organizations, cities and regions; inaccessibility of the study for some patients on mechanical ventilation; high need for CT studies to diagnose other diseases.

At the same time, a comprehensive assessment of anamnestic, clinical and radiological data makes it possible to identify a clinically confirmed case of COVID-19, refer the patient and begin antiviral therapy.

In this regard, CT can be a "first-line" study in those medical organizations/territories that have a sufficient number of devices and staffing to perform the required volume of research without compromising the timely diagnosis of other diseases (oncological, neurological, etc.) in patients most in need of this study.

Ultrasound examination (USE) of the lungs in patients with suspected COVID-19 pneumonia is an additional imaging method that does not replace radiography and computed tomography. If the correct technique is followed, the correct indications are selected and the presence of trained medical personnel, this study is highly sensitive in detecting interstitial changes and consolidations in the lung tissue, but only when they are located subpleurally. Ultrasound data do not allow one to unambiguously determine the cause and/or the actual extent of changes in the lung tissue.

Electrocardiography (ECG) in standard leads is recommended for all patients. Because it is known that viral infection increases the risk of developing rhythm disturbances and acute coronary syndrome.

Basic principles of COVID-19 therapy

Antiviral therapy includes the use of the following drugs: favipiravir, remdesivir, umifenovir, oseltamivir, hydroxychloroquine, interferon-alpha.

Favipiravir is a synthetic antiviral drug, a selective inhibitor of RNA polymerase, active against RNA viruses. It uses for patients with moderate or critical disease of coronavirus infection.

Chloroquine (hydroxychloroquine) is a drug used to treat malaria and some other protozoal infections. The mechanism of action of the drug against viral infections is not fully understood. The published literature indicates several options for its effect on the COVID-19 virus. This is an obstacle to the penetration of the virus into the cell and its replication. The drug is used to treat malaria and some

systemic connective tissue diseases, and is also considered as a drug for the treatment of COVID infection. Compared to chloroquine, hydroxychloroquine has less cytotoxic and more pronounced antiviral effects. These drugs are used to treat patients with moderate severity of the disease.

Remdesivir is prescribed for severe or critical cases of COVID infection.

Recombinant interferon alpha (IFN-α) is used intranasally and has immunomodulatory, anti-inflammatory and antiviral effects. Its mechanism of action is based on preventing the replication of viruses that enter the body through the respiratory tract. Pregnant women are prescribed only recombinant IFN-α2b.

Glucocorticosteroids (dexamethasone) are the drugs of choice for the treatment of patients with coronavirus infection. They inhibit all phases of inflammation, the synthesis of a wide range of pro-inflammatory mediators, an increase in the concentration of which as part of a cytokine storm is associated with an unfavorable prognosis for COVID-19 and the risk of developing ARDS and sepsis.

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Receptor inhibitors (tocilizumab, levilimab, olokizumab, sarilumab, tocilizumab, canakinumab) are prescribed in the presence of pathological changes in the lungs, moderate/severe pneumonia after an X-ray examination and computed tomography scan (in the presence of confluent infiltration-type darkening - a "white lung" symptom).

Anticoagulants (rivaroxaban) are prescribed to prevent thromboembolism of the deep veins and pulmonary artery.

Oxygen therapy is used in cases of acute respiratory failure.

Antibacterial therapy is prescribed only if:

- convincing signs of a bacterial infection,
- appearance of purulent sputum, leukocytosis > 12×109/l (in the absence of previous use of glucocorticoids), increase in the number of band neutrophils more than 10 %).

The vast majority of patients with COVID-19, especially those with moderate disease, do not require antibacterial therapy.

Antibacterial therapy is prescribed only if:

- when a bacterial infection occurs,
- the appearance of purulent sputum if leukocytosis > 12×109/l (in the absence of previous use of glucocorticoids and an increase in the number of band neutrophils by more than 10 %).

The vast majority of patients with COVID-19, especially those with mild to moderate disease, do not require antibiotic therapy.

Immunotherapy includes the use of anti-Covid plasma; COVID globulin; monoclonal antibodies. The COVID-globulin drug is a highly purified immunoglobulin G preparation that has antibody activity against SARS-CoV-2. 1 ml of the drug contains active ingredient: human plasma proteins, of which immunoglobulin G is at least 95 % – 100 mg.

Dosage: the drug is administered intravenously by drip without dilution in a single dose of 4 ml/kg body weight.

Recommended for use in patients at high risk of severe disease:

- age over 65 years,
- presence of concomitant diseases (diabetes mellitus, obesity, chronic diseases of the cardiovascular system).

Monoclonal antibodies to the S protein of SARS-CoV2. Currently, single-component (sotrovimab, regdanvimab), as well as combination (bamlanivimab + etesevimab; casirivimab + imdevimab) drugs are used.

The use of monoclonal antibodies is recommended in inpatient settings, as well as in day hospital settings no later than 7 days from the onset of the disease.

Specific prevention.

Today, there are several types of vaccines to prevent SARS-CoV-2:

1. Vector vaccines (recombinant) - for preparation they use a modified virus (vector – adenovirus), which has built-in "instructions" for the production of the SARS-CoV-2 spike protein. The vector delivers it into the cell, the immune system recognizes the antigen, and in response produces antibodies and T cells. (Oxford AstraZeneca, Janssen (Johnson & Johnson), Cansino.

2. Inactivated vaccines - contain a killed virus. The body reacts to it and produces antibodies. (CoronaVac Sinovac, SinoPharm, Bharat Biotec (Covaxin), Valneva.

3. RNA vaccines – contain mRNA molecules that encode the coronavirus spike protein, thereby promoting the synthesis of antibodies (Pfizer BioNTech, Moderna).

4. Subunit vaccines contain components (antigens) of the virus that best stimulate an immune response. For example, a vaccine based on protein subunits, which contains the spike protein of the SARS-CoV-2 molecule (Novavax, Sanofi).

The World Health Organization has approved for emergency use the vaccines that are available in Ukraine:

AstraZeneca is a vector vaccine developed by the Swedish-British company AstraZeneca together with the University of Oxford. The AstraZeneca vaccine is produced in the European Union, Great Britain, and the Republic of Korea. The vaccine, manufactured under license in India, is called Covishield.

To be fully immunized, you need to receive two doses of the vaccine 12 weeks apart. The first and second doses are available with the AstraZeneca vaccine, produced at various sites, including sub-licensed production in India. After all, the composition and manufacturing technology of the vaccine are identical.

Comirnaty/Pfizer-BioNTech is an mRNA vaccine against COVID-19, developed by the German biotechnology company BioNTech together with the American pharmaceutical concern Pfizer. For full immunization, you need to receive 2 doses 21–28 days apart. Approved by the World Health Organization for emergency use.

The Moderna vaccine is an mRNA vaccine developed by the American pharmaceutical company Moderna.

The drug is included in the WHO list of vaccines for emergency use, and is also authorized for emergency use in the USA, UK, Canada, EU and other countries.

The vaccine is stored as a frozen suspension at –25 ºC to –15 ºC in a multi-dose vial. Vials can be stored in the refrigerator at 2–8 °C for 30 days.

The Moderna COVID-19 vaccine is administered intramuscularly. To be fully immunized, you need to receive two doses of the vaccine 28 days apart.

CoronaVac / Sinovac Biotech is an inactivated vaccine developed by a Chinese biopharmaceutical company engaged in the research, development, production and marketing of vaccines to protect people from infectious diseases. To be fully immunized, you must receive two doses 14 to 28 days apart. The vaccine has been approved by WHO for emergency use.

Nonspecific prevention:

Measures regarding the source (patient) of infection:

- isolation of patients in boxed rooms/wards of an infectious diseases hospital;
- use of masks for patients, which must be changed every 2 hours,
- transportation of patients with special transport,
- observance of cough hygiene by patients,
- use of disposable medical instruments.

Measures aimed at the mechanism of transmission of the infectious agent:

- hand washing,
- use of medical masks,
- use of special clothing for health workers,
- carrying out disinfection measures,
- ensuring air disinfection,
- disposal of class B waste.

Activities aimed at susceptible populations:

- elimination therapy, which is irrigation of the nasal mucosa with an isotonic sodium chloride solution, reduces the number of both viral and bacterial pathogens of infectious diseases
- use of drugs for topical use with barrier functions.
- timely access to medical institutions for medical help in the event of acute respiratory viral infection symptoms is one of the key factors in the prevention of complications.

Theoretical questions:

1. Characteristics of pathogens of coronavirus infection.
2. Morphology and antigenic structure of the SARS-CoV-2 virus.
3. Features of replication of the SARS-CoV-2 virus.
4. Epidemiology, pathogenesis and main clinical forms of COVID-19.
5. Features of clinical manifestations of COVID-19 in the elderly, children and pregnant women.
6. Laboratory diagnostic methods for COVID-19.
7. Treatment methods for COVID-19.
8. Specific and nonspecific prevention of COVID-19.

Practical tasks performed in class:

1. Study of demonstration drugs.
2. Analysis of the laboratory diagnostics scheme for COVID-19.
3. Sketching the morphological structure of the SARS-CoV-2 virus in the protocol.
4. Drawing up the protocol.

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Навчальне видання

ЛАБОРАТОРНА ДІАГНОСТИКА КОВІДНОЇ ІНФЕКЦІЇ

Методичні вказівки
для студентів II–III курсів
медичного та стоматологічного факультетів
з дисципліни «Мікробіологія, вірусологія та імунологія»

Упорядники: Мішина Марина Мітрофановна
Коцар Олена Василівна
Кочнєва Олена Володимирівна
Ковальов Максим Михайлович
Калашник-Вакуленко Юлія Михайлівна

Відповідальний за випуск: О. В. Коцар

Комп'ютерний набір О. В. Коцар
Комп'ютерна верстка О.Ю. Лавриненко

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Редакційно-видавничий відділ
ХНМУ, пр. Науки, 4, м. Харків, 61022
izdatknmurio@gmail.com, vid.redact@knmu.edu.ua

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