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April 28, 2020

INSTRUCTION

Kit for Qualitative Detection of Coronavirus RNA (*SARS-CoV-2*) by Real-Time RT-PCR «CoV-2-Test»

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Version 2 – revised on April 28, 2020

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Introduction

Early diagnosis of severe acute respiratory infection (atypical pneumonia) caused by SARS-CoV-2 coronavirus is extremely important for correct and timely diagnosis and treatment.

The target analyte detected with using the kit «CoV-2-Test» is a specific part of the genomic RNA of the SARS-CoV-2 strain (2019-nCoV, COVID-19) of the coronavirus, a fragment of N gene encoding the nucleocapsid phosphoprotein.

RNA samples extracted from a patient's biological material serve as **the material for the study.**

The scientific validity of the target analyte lies in its specificity (uniqueness of RNA/DNA sequence) in relation to the genome of the SARS-CoV-2 strain (2019-nCoV, COVID-19) of the coronavirus. The proposed fragment of the coronavirus genome as a target analyte is recommended by the FDA.[1]

The new SARS-CoV-2 coronavirus is a single-stranded RNA-containing virus belonging to the family Coronaviridae, belonging to the Beta-CoV b lineage. The virus is classified as group II pathogenicity, as well as some other representatives of this family (*SARS-CoV*, *MERS-CoV*) and is presumably a recombinant virus between the bat coronavirus and an unknown origin of the coronavirus[2].

Application area: clinical laboratory diagnostics, infectious diagnostics.

Indications and Contraindications for Use

Detection of *SARS-CoV-2* RNA by PCR is recommended for use in patients with clinical symptoms of respiratory disease suspected of infection caused by *SARS-CoV-2*, especially in those patients arriving from epidemiologically disadvantaged regions immediately after their initial examination, as well as in contact persons[2].

The applied RNA determination method refers to non-invasive procedures, does not pose a threat to human health and does not cause complications.

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[1] Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primer and Probe Information (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

[2] Temporary guidelines "Prevention, Diagnosis and Treatment of a Novel Coronavirus Infection (COVID-19)", Version 4 (27.03.2020) (Ministry of Health of the Russian Federation).

1. Intended use

1.1. Intended use: the kit «CoV-2-Test» is intended to detect *SARS-CoV-2* coronavirus RNA by single-step reverse transcription – multiplex allele-specific polymerase chain reaction with hybridization-fluorescent detection in RNA samples.

1.2. Number of reactions

The kit is designed for 96 reactions.

1.3. Application area: clinical laboratory diagnostics, infectious diagnostics.

1.4. Qualifications of user: for professional use in medical institutions and clinical diagnostic laboratories. Professional level of potential consumers – doctor of clinical laboratory diagnostics, medical laboratory technician.

1.5. Functional purpose. Obtained results can be used to support the diagnosis of severe acute respiratory viral infection caused by *SARS-CoV-2* coronavirus.

1.6. Indications for use

Detection of *SARS-CoV-2* RNA by PCR is recommended for use in patients with clinical symptoms of respiratory disease suspected of infection caused by SARS-CoV-2, especially in those patients arriving from epidemiologically disadvantaged regions immediately after their initial examination, as well as in contact persons[2].

1.7.Demographic and population aspects of application: without restrictions.

1.8. Contraindications for use: No.

1.9. Sterility: the product is not sterile.

2. Operating Principle

Method

Single-step reverse transcription - multiplex allele-specific polymerase chain reaction (RT-PCR) in real time with hybridization-fluorescent detection.

Test sample type

Biological materials for research are:

1. pharynx (oropharynx) smear
2. sputum
3. nasal smear

Detection Principle

Detection of nucleic particles of *SARS-CoV-2* coronavirus is based on the use of a single-step reverse transcription reaction followed by a real-time polymerase chain reaction in a single tube.

The RT-PCR buffer consists of all main reagents, including "warm start" reverse transcriptase, "hot start" heat stable DNA polymerase, dNTPs, and optimized buffer.

Primer-mix contains fluorescently labeled oligonucleotide probes that hybridize with a complementary section of the amplified target DNA and destroyed by Taq polymerase, resulting in the separation of the dye and quencher, and increase in the intensity of fluorescence. This allows accumulation of a specific amplification product to be recorded by measuring the intensity of the fluorescent signal in «real time» mode.

The kit contains reagents for multiplex RNA detection of a fragment of the *SARS-CoV-2* coronavirus N gene and an internal control sample RNA fragment (hereinafter «ICS»): amplification products of the *SARS-CoV-2* coronavirus N gene are registered via a channel corresponding to FAM fluorophore, and ICS amplification products are registered by HEX.

ICS allows evaluating the effectiveness of RNA isolation and possible presence of inhibitors in the sample, the presence of which can lead to false negative results.

Method limitation

A possible reason for obtaining a false positive result is contamination at the stage of RNA isolation or real-time RT-PCR reaction. A false positive result can be detected using a negative control sample.

Breach of the package integrity during transportation.

Use of an expired kit.

Violation of storage and transporting conditions of samples.

The time of RT-PCR reaction is 2 hours (excluding sample preparation).

3. Reagent Kit Composition

Versions

The kit «CoV-2-Test» is available in two versions:

1) Version 1.

Composition: Reagent kit for RT-PCR with 5x buffer «CoV-2-Test-RT-PCR-r».

2) Version 2.

Composition: Reagent kit for RT-PCR with 5x buffer «CoV-2-Test-RT-PCR-r», reagent kit for RNA extraction «CoV-2-Test-extraction».

Number of Tests

Each reagent kit for RT-PCR «CoV-2-Test-RT-PCR-r» is designed to conduct 96 RT-PCR reactions that corresponds to testing of 94 test samples, negative and positive control samples, or 32 single setups of the test samples with negative and positive control samples in each setup.

Each reagent kit for RNA extraction («CoV-2-Test-extraction») is designed for isolation of RNA from 96 samples with a volume of 100 µl.

Kit Composition

Table 1 – Version 1. Composition of «CoV-2-Test» reagent kit:

#	Reagent Name	Cap Color	Description	Quantity, Volume
RT-PCR reagent kit with 5x buffer «CoV-2-Test-RT-PCR-r»				
1	RT-PCR-buffer-r	brown	Transparent colorless liquid	1 tube, 480 µl
2	Primer mix	colorless	Transparent liquid of pink color	1 tube, 480 µl
3	PC	red	Transparent colorless liquid	1 tube, 480 µl
4	NC	blue	Transparent colorless liquid	1 tube, 1500 µl
5	Internal control sample (ICS)	yellow	Transparent colorless liquid	1 tube, 940 µl

Table 2 – Version 2. Composition of the kit «CoV-2-Test»:

#	Reagent Name	Cap Color	Description	Quantity, Volume
RT-PCR reagent kit with 5x buffer «CoV-2-Test-RT-PCR-r»				

#	Reagent Name	Cap Color	Description	Quantity, Volume
1	RT-PCR-buffer-r	brown	Transparent colorless liquid	1 tube, 480 µl
2	Primer mix	colorless	Transparent liquid of pink color	1 tube, 480 µl
3	PC	red	Transparent colorless liquid	1 tube, 480 µl
4	NC	blue	Transparent colorless liquid	1 tube, 1500 µl
5	Internal control sample (ICS)	yellow	Transparent colorless liquid	1 tube, 940 µl
Reagent kit for RNA extraction «CoV-2-Test-extraction»				
1	Lysing buffer	-	Transparent liquid	1 bottle, 48 ml
2	Washing solution	-	Transparent liquid	2 bottles, 68 ml each
3	Eluent	-	Transparent liquid	1 bottle, 101 ml
4	Magnetic microbeads	-	Suspension. Brown colored after shaking	1 tube, 960 µl
5	Additional component for lysis	-	Transparent liquid	1 tube, 960 µl

The kit for RNA extraction «CoV-2-Test-extraction» contains a lysing buffer, additional component for lysis, washing solution, eluent, suspension with magnetic particles. All components are ready to use.

RT-PCR buffer-r is ready for use and contains reverse transcriptase with suppressed RNase H activity, “hot start” heat stable DNA polymerase, dNTP, and a buffer.

Reverse transcriptase is ready to use. It is a solution of enzyme in a storage buffer.

Primer-mix is ready to use, contains a multiplex mix of primers and probes:

1. Primers and probe for the N gene fragment encoding the nucleocapsid phosphoprotein, which is a specific part of the genomic RNA of the *SARS-CoV-2* coronavirus strain. The N gene fragment used as a target in this test system is recommended by FDA for detecting coronavirus. [1] Detection is performed via FAM channel.

2. Primers and probe to internal control sample. Detection via HEX channel.

Reaction via HEX indicates sufficient efficiency of nucleic acid extraction and absence of PCR inhibitors. In the absence of reaction, the result should be considered false, and in this case, it is recommended to re-isolate RNA for PCR-testing for this test sample.

Positive control sample (PC) is ready for use and represents plasmid vector with synthetic DNA inserts, complementary RNA of internal control sample and N gene fragment of the coronavirus (*SARS-CoV-2*).

Negative control sample (NC) is ready for use and represents RNase free deionized sterile water.

Internal control sample (ICS) contains RNA packed in a protein envelope in buffer solution.

The kit contains no medicines for medical use, substances of human or animal origin.

4. Kit Characteristics

4.1 Technical and Functional Characteristics

Table 1.1. «CoV-2-Test» kit.

Version 1

Parameter Name	Characteristics and Standards	Tech Spec.(TS) paragraph
Version 1 of Kit «CoV-2-Test»		
1. Technical Characteristics		**1**
1.1. Visual appearance		
Reagent kit for RT-PCR with readymade 5x buffer «CoV-2-Test-RT-PCR-r»		
RT-PCR-buffer-r	Transparent colorless liquid	1.2
Primer-mix	Transparent liquid of pink color	1.2
PC	Transparent colorless liquid	1.2
NC	Transparent colorless liquid	1.2
Internal Control Sample, ICS	Transparent colorless liquid	1.2

1.2. Completeness	In accordance with p.1.4 TS 21.20.23-015-97638376-2020	1.4
1.3. Labeling	In accordance with p. 1.5 TS 21.20.23-015-97638376-2020	1.5
1.4. Packaging	In accordance with p. 1.6 TS 21.20.23-015-97638376-2020	1.6
2. Functional Characteristics		5.3.1.1
Reagent kit for RT-PCR with 5x buffer «CoV-2-Test-RT-PCR-r»		
Positive result with PC	Detection of the increase of fluorescence signal in test tubes with PC via FAM Ct≤35 channel and via HEX Ct≤35 channel.	5.3.1.1 A1
Reaction with ICS	Detection of the increase of fluorescence signal in test tubes with ICS via HEX Ct≤35 channel	5.3.1.1 A2
Negative result with NC	In test tubes with NC via FAM and HEX channel, Ct is not shown or >45.	5.3.1.1 A3
3. Analytical Characteristics		5.3.1.2
RT-PCR reagent kit with readymade 5x buffer «CoV-2-Test-RT-PCR-r»		
Analytical specificity	It is specific to N gene fragment encoding the nucleocapsid phosphoprotein - a specific part of the *SARS-CoV-2* coronavirus strain genomic RNA and RNA fragment of Internal Control Sample. There were no false positive results when testing NA samples of severe acute respiratory syndrome *(SARS-Cov)* or Middle East respiratory syndrome *(MERS-Cov)*.	5.3.1.2 A1
Analytical sensitivity	At least 500 copies of ICS per 1 ml via HEX channel Ct≤40 At least 500 copies of PC per 1 ml via HEX channel Ct≤40 and via FAM channel Ct≤40	5.3.1.2 A2

Table 1.2. Reagent kit «CoV-2-Test». **Version 2**

Parameter Name	Characteristics and standards	Tech Spec.(TS) paragraph

Version 2 of Reagent Kit «CoV-2-Test»		
1. Technical Characteristics		**1**
1.1 Visual appearance		
RT-PCR reagent kit with 5x buffer «CoV-2-Test-RT-PCR-r»		
RT-PCR buffer-r	Transparent colorless liquid	1.2.
Primer-mix	Transparent liquid of pink color	1.2.
PC	Transparent colorless liquid	1.2.
NC	Transparent colorless liquid	1.2.
Internal Control Sample, ICS	Transparent colorless liquid	1.2.
Reagent kit for RNA extraction «CoV-2-Test-extraction»		
Lysing buffer	Transparent liquid	1.2.
Washing solution	Transparent liquid	1.2.
Eluent	Transparent liquid	1.2.
Additional component for lysis	Transparent liquid	1.2.
Magnetic microbeads	Suspension. Brown-colored after shaking.	1.2.
1.2. Completeness	In accordance with p. 1.4 TS 21.20.23-015-97638376-2020	1.4
1.3. Labeling	In accordance with p. 1.5 TS 21.20.23-015-97638376-2020	1.5
1.4. Packaging	In accordance with p. 1.6 TS 21.20.23-015-97638376-2020	1.6
2. Functional Characteristics		**5.3.2.1**
Reagent kit for RT-PCR with 5x buffer «CoV-2-Test-RT-PCR-r»		
Positive result with PC	Detection of the increase of fluorescence signal in test tubes with PC via FAM Ct≤35 channel and via HEX Ct≤35 channel.	5.3.2.1 A1
Reaction with ICS	Detection of the increase of fluorescence signal in test tubes with ICS via HEX Ct≤35 channel	5.3.2.1 A2
Negative result with NC	In test tubes with NC via FAM and HEX channel, Ct is not shown or >45.	5.3.2.1 A3

Reagent kit for RNA extraction «CoV-2-Test-extraction»		
Extraction efficiency	Confirmed with ICS at least 500 copies per ml via HEX channel Ct≤40	5.3.2.1 B1
3. Analytical Characteristics		**5.3.2.2**
RT-PCR reagent kit with 5x buffer «CoV-2-Test-RT-PCR-r»		
Analytical specificity	It is specific to N gene fragment encoding the nucleocapsid phosphoprotein - a specific part of the *SARS-CoV-2* coronavirus strain genomic RNA and RNA fragment of Internal Control Sample. There were no false positive results when testing NA samples of severe acute respiratory syndrome *(SARS-Cov)* or Middle East respiratory syndrome *(MERS-Cov)*.	5.3.2.2 A1
Analytical sensitivity	At least 500 copies of ICS per 1 ml via HEX channel Ct≤40 At least 500 copies of PC per 1 ml via HEX channel Ct≤40 and via FAM channel Ct≤40	5.3.2.2 A2

4.3 Clinical Efficiency

Diagnostic specificity is established in clinical trials.
Diagnostic sensitivity is established in clinical trials.

5. Risks Associated With the Use of Reagent Kit «CoV-2-Test»

The border risk zone includes the following:

1. loss of functional properties of reagents included in the kit due to transportation, storage or operation under inappropriate conditions,
2. contamination of reaction mixtures with RNA samples under study with a PC tube content or PCR products,
3. a test is performed using a low-quality RNA sample extracted from peripheral blood plasma (low concentration and/or poor purification),
4. failure to meet the requirements for sample preparation, testing and disposal due to unskilled personnel works with the kit;
5. use of unsuitable kit (use after the expiration date or if the packaging is broken).

In the area of the unacceptable zone, no risks were identified.

Total residual risk of using a medical product kit of reagents for qualitative detection of coronavirus RNA (*SARS-CoV-2*) by real-time RT-PCR "CoV-2-Test" is acceptable, the benefit of its use exceeds the risk.

6. Precautions measures

Potential risk Class III – in accordance with Nomenclature Classification of Medical Devices approved by the Order of the Ministry of Health of the Russian Federation dated June 6, 2012 No.4n.

All components and reagents included in the kit of reagents "CoV-2-Test" belong to Hazard Class 4 (low-hazard substances) in accordance with State Standard GOST 12.1.007-76 "Safety Standards System. Harmful Substances. Classification and General Safety Requirements".

The reagents included in the «CoV-2-Test» kit have a low vapor elasticity and exclude the possibility of inhalation poisoning.

The reagents included in the «CoV-2-Test» kit are non-toxic, since they are prepared by mixing separate non-toxic components.

Handling material infected or suspected of being infected with *SARS-CoV-2* coronavirus is carried out in accordance with the requirements of sanitary and epidemiological rules for the safety when working with microorganisms in pathogenicity (hazard) groups 3 and 4[3] (Health Regulations 1.3.3118-13), Methodology Guidelines "Organization of Laboratory Work Using Methods of Nucleic Acid Amplification When Working With Material Containing Microorganisms of Pathogenicity Groups I-IV" (Methodology Guidelines 1.3.2569-09), temporary guidelines "Prevention, Diagnosis and Treatment of a Novel Coronavirus Infections *(2019-nCoV)*, Version 3 of 03.03.2020 (Approved by the Ministry of Health of the Russian Federation and Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor)), and Information letter from Rospotrebnadzor dated 21.01.2020 No. 02/706-2020-27 "Temporary Recommendations for Organization of Laboratory Diagnostics of a Novel Coronavirus Infection (*2019-nCoV*)".

[3] Temporary guidelines "Prevention, Diagnosis and Treatment of Novel Coronavirus Infection (COVID-19)", Version 4 (27.03.2020) (Ministry of Health of the Russian Federation).

Personnel should ensure and comply with the rules of biological safety and requirements for organization of work and conduct it in order to prevent contamination of premises and equipment with nucleic acids and(or) amplicons of tested samples.

The work should be carried out in a laboratory that performs molecular biological (PCR) studies of clinical material in compliance with the Sanitary and Epidemiological Rules and Regulations SanPiN 2.1.7.2790-10 “Sanitary and Epidemiological Requirements for Medical Waste Handling”. Personnel should follow recommendations set out in Methodology Guidelines 287-113, Methodology Guidelines 1.3.2569-09.

When working it is required:

- remove unused reagents in accordance with paragraph 4.28 of SanPiN 2.1.7.2790-10 “Sanitary and Epidemiological Requirements for Medical Waste Handling”.

ATTENTION! When removing waste after amplification (tubes with PCR products), it is not allowed to open tubes and spill the content, since this can lead to contamination of a laboratory area, equipment and reagents with PCR products;

- use the kit strictly for its intended purpose, according to this instruction;
- only specially trained personnel is allowed to work with the kit (a specialist with higher medical education who has been trained in licensed qualification courses to work with Pathogenic Biological Agents (PBA) of pathogenicity groups III and IV and to conduct PCR diagnostics, a laboratory assistant with secondary special medical education);
- do not use the kit after the expiration date;
- avoid contact with skin, eyes and mucous membrane. In case of contact, immediately flush the affected area with water and seek medical attention.

The precautions are not provided for the effects of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure drops, overloads, or sources of thermal ignition.

The kit contains no substances of human or animal origin that have a potential infectious nature, so precautions against any special, unusual risks when using or selling the product are not provided.

7. Equipment and Materials

Work with RNA extraction kit «CoV-2-Test-extraction» is carried out in working area 2 (for NA isolation), work with a reagent kit for RT-PCR with 5x buffer «CoV-2-Test-RT-PCR-r» is carried out in working area 3 (for preparing reactions) (Methodology Guidelines 1.3.2569-09).

Equipment for RNA Extraction:

1. PCR-box, biological safety cabinet Class II and III (for example, «BMB-II – «Laminar-S-1,2», «Laminar Systems", Russia).
2. Vortex (for example, «TETA-2», «Biokom», Russia).
3. Microcentrifuge for Eppendorf tubes with volume for 1.5 ml, acceleration of at least 10,000 g during extraction using centrifugation;
4. Thermostat for Eppendorf tubes with volume for 1.5 ml with heating possibility at least up to 80°C;
5. Medical vacuum aspirator with trap flask;
6. Dispensers of variable type, mechanical or electronic;
7. Rack for tubes (1.5 ml);
8. A separation magnetic rack for 1.5 ml Eppendorf tubes;
9. Refrigerator for 2 to 8 °C.

Equipment for RT-PCR Reaction:

1. PCR-box, biological safety cabinet Class II and III (for example, «BMB-II – «Laminar-S-1,2», «Laminar Systems", Russia).

2. Vortex (for example, «TETA-2», «Biokom», Russia).

3. Kit of electronic or automatic variable volume dispensers (e.g., Eppendorf, Germany).

4. Refrigerator for 2 °C to 8 °C and a freezer for at least - 16 °C.

5. A cycler[4] of plate type, for example, CFX96 (BioRad, USA), "DTprime", "DTlight" ("DNA-Technology", Russia) or cycler of rotary type, e.g., "Rotor-Gene" 3000 or 6000 (Corbett Research, Australia) or equivalent.

Materials and reagents not included in the product:

[4] Amplifiers must be maintained, calibrated, and used in accordance with the manufacturer's recommendations. The use of this kit while a device out of calibration may have an impact on the performance of the test.

ATTENTION! When working with RNA, it is required to use only disposable sterile plastic consumables that have a special marking «RNase-free».

1. Disposal tips with an aerosol barrier up to 1,000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA).
2. Disposal or individual coat and disposable talc-free gloves.
3. Container with disinfectant.
4. "Workplace" racks for 0.2 ml tubes or for 0.2 ml strip tubes (e.g., "InterLabService", Russia).

8. Test Samples

Test sample type

Biological materials for research are:

1. pharynx (oropharynx) smear
2. sputum
3. nasal smear

8.1 Biological Sample Collection

Attention! Before starting work, it is required to study Guidelines "Sampling, Transportation and Storage of Clinical Material for PCR-Diagnostics", developed by the Federal State Budgetary Institution of Science Central Research Institute of Epidemiology of Federal service for surveillance on consumers' rights protection and human well-being (Rospotrebnadzor), Moscow, 2012**.**

Biological material is collected and packed by healthcare organization specialist specially trained to follow biological safety requirements and rules when working and collecting material suspected of being infected with microorganisms of pathogenicity group III.

Collection of Material for Testing

Nasal and pharynx (oropharynx) smear

Nasal smear is collected using dry sterile cotton sponge with plastic basis. Sponge is entered by light moving along outer dorsum of the nose 2-3 cm deep till lower scroll bone. Then sponge is moved down and entered in lower nasal passage, rotary moved and removed along outer dorsum of the nose.

Oropharynx smear. Swabs are collected using dry sterile cotton sponge with plastic basis by rotate moving from tonsils surface, faucial pillars and back oropharyngeal wall.

After material sampling sponge (work part of probe with cotton sponge) is placed in disposable sterile Eppendorf tube, containing transport medium (or 500 ml of sterile saline solution) and carefully break off the plastic stick at a distance of no more than 0.5 cm from the working part, leaving the working part of the probe with the material inside. The test tube is tightly closed with a cap.

Sputum.

Material sampling (not less than 1 ml) is performed after pre-rinsing a mouth with water in disposable graduated sterile containers with wide screw cap of 50 ml volume.

Transportation, Storage, Disposal of Clinical Material:

Recording, storage, transfer and transportation of biological material suspected to be causative agent of severe acute respiratory infections COVID-19, should be performed in accordance with applicable sanitary and epidemiological rules for the safety when working with microorganisms in pathogenicity (hazard) groups 3 and 4 (Health Regulations 1.3.3118-13), and current sanitary regulations on recording, storage, transfer and transportation of material containing microorganisms I-IV pathogenicity groups.

Transportation of biomaterial to laboratory and its storage is carried out in accordance with requirements of temporary guidelines "Prevention, Diagnosis and Treatment of Novel Coronavirus Infection (*2019-nCoV*)" (Temporary Methodology Guidelines *2019-nCoV*) and "Temporary Guidelines for Laboratory Diagnostics of Novel Coronavirus 2019 (*2019-nCoV*) via polymerase chain reaction (PCR)" (Table 4)[5].

ATTENTION! Avoid repeated freezing and thawing of samples.

Utilization of clinical material (Class B) as extremely epidemiologically hazardous waste is carried out in accordance with SanPiN 2.1.7.2790-10.

[5] Temporary Recommendations for Organization of Laboratory Diagnostics of a Novel Coronavirus Infection (2019-nCoV) No. 02/706-2020-27 of 21.01.2020. – Rospotrebnadzor.

Table 4 – Biomaterial Storage and Transportation Conditions

Sample type	**Material collection requirements**	**Transportation**	**Storage conditions prior transporting**	**Comments**
Nasaland pharynx (oropharynx) smear	Plastic tubes and sponges for smear	4°C	<= 5 days: 4°C > 5 days[6]: -70°C	Nasopharynx and pharynx sponges should be placed in a single tube to increase viral load
Sputum	Sterile container	4°C	<= 48 hours: 4°C > 48 hours: -70°C	Make sure that the material is coming from the lower respiratory tract

8.2. Clinical Material Preparation[7].

1. Nasal and pharynx (oropharynx) smear.

- Transfer epithelial cells scraping using a disposable probe to 1.5 ml plastic tube with transport medium (or 500 µl of sterile saline), and mix carefully.
- Remove the probe by pressing it against the tube wall and squeezing out the excess liquid. Close the tube tightly.
- Centrifuge the test tube at 13,000 rpm for 10 minutes.
- Remove supernatant liquid, leaving approximately 100 µl (sediment +liquid fraction) in the tube.

2. Sputum.

- Liquefy the material using Mucolysin in a 5:1 ratio (5 parts of Mucolysin to 1 part of sputum).
- Close the container cap, shake the contents and incubate for 20-30 minutes at room temperature, shaking periodically.
- Use a dispenser to collect 1 ml of liquefied sputum into a 1.5 ml plastic Eppendorf tube and centrifuge at 5,000-7,000 g for 10 minutes.

[6] To transport samples, a vehicle (transport medium) containing antifungal and antibiotic additives is used.

[7] Methodology Guidelines "Organization of Laboratory Work Using Methods of Nucleic Acid Amplification When Working With Material Containing Microorganisms of Pathogenicity Groups I-IV". Moscow: 2009

- Remove 800 µl of the supernatant liquid, mix the remaining cells with 200 µl of the remaining liquid.

- It is allowed to separate from 100 µl of liquefied sputum without centrifugation.

8.3 Interfering substances and restrictions on the use of testing material

The effect of potentially interfering substances on the CoV-2-Test reagent kit was tested for potentially interfering substances that will occur during the biological material sampling procedure:

1. Hemoglobin
2. Mucin
3. Ibuprofen
4. Ambrobene
5. Bromhexin
6. Kaletra
7. Interferon
8. TeraFlu

Based on the results of the study, potentially interfering substances encountered during RNA isolation from clinical material, evaluated at concentrations that are expected to occur with normal use of the «CoV-2-Test» kit, do not affect the test result.

Restrictions on the use of the tested material:

- testing material cannot be used in case of violation of storage and transportation conditions (temperature, duration, multiple freezing-thawing);

- it is not allowed to use plasma samples contaminated with clots of white blood cells or red blood cells during separation;

- samples contaminated with foreign biological material are not allowed to be used.

9. Testing

It is not required to install, assemble, adjust, or calibrate a medical device before commissioning.

ATTENTION! Make sure that an internal control sample (ICS) has been added to the test biomaterial before RNA isolation.

ATTENTION! When working with RNA, it is required to use only disposable sterile plastic consumables that have a special marking

«RNase-free». It is required to use a separate tip with an aerosol barrier for each reaction component.

ATTENTION! Components of reaction mixture should be mixed according to Table 6 directly in PCR tubes before testing.

9.1 Isolation of RNA from Biological Material

Suspension of magnetic microbeads is biphasic, easily and quickly forms clearly separated two phases. Before the work and before each manipulation with magnetic microbeads suspension complete re-suspension of the solution on vortex or by pipetting.

All components of the kit should be thoroughly mixed before use.

RNA Isolation Protocol Using Reagent Kit for RNA Extraction («CoV-2-Test-extraction»):

1. Preparation of Reagents:

1.1. Get tubes with NC and ICS from RT-PCR kit with 5x buffer «CoV-2-Test-RT-PCR-r», and thaw at room temperature.

1.2. Carefully mix the lysing buffer, flushing solution and eluent by inverting it 10 times.

1.3. Mix additional component for lysis, precipitate drops on the vortex.

1.4. Fully re-suspend the tube content with magnetic microbeads on the vortex. Spill drops from a test tube walls, and then mix further by pipettingup and down using a dispenser.

1.5. Prepare a mixture of lysis buffer, additional reagent for lysis and magnetic microbeads in a separate tube, adding amounts based on one sample: 10 µl – additional solution for lysis, 10 µl of magnetic microbeads solution and 500 µl of lysis buffer, taking into account the stock for 1 sample more. Mix the mixture on the vortex.

Note: thoroughly mix the obtained mixture by shaking, store for no more than a month at a temperature of +2 to +8°C.

2. RNA Extraction

2.1. Lable the required number of disposable test tubes with 1.5 ml volume for test samples, label one tube for NC.

2.2. Add to each tube test samples and 100 μl[8] of NC (from RT-PCR kit with 5x buffer «CoV-2-Test-RT-PCR-r»), using a separate tip with a filter for each sample.

2.3. Add to each test sample 10 μl of ICS from RT-PCR kit with 5x buffer «CoV-2-Test-RT-PCR-r»), and 520 μl of lysing components and magnetic particles mixture.

2.4. Add 520 μl mixture of lysing components and magnetic microbeads to NC tube. ICS is not added into NC tube.

2.5. Warm test tubes at 60 °C for 10 minutes, mix on the vortex for 10-15 seconds and precipitate the drops by short centrifugation.

2.6. Place tubes in a magnetic rack for 1.5-2 min[9], remove supernatant liquid using a dispenser or aspirator.

2.7. Add 700 μl of flushing solution, tightly close caps, mix on the vortex and precipitate drops by short centrifugation.

2.8. Place test tubes with samples in a magnetic rack for 1.5-2 min., remove supernatant liquid.

2.9. Leave the tubes in the magnetic rack, add 800 μl of eluent to the tubes, remove supernatant liquid not stirring the tubes content.

2.10. Add 50 μl of eluent, close the tube caps tightly and mix on the vortex.

2.11. Place the test tubes in thermostat at a temperature of 80°C for 5 minutes, stirring every 2 minutes.

2.12. Precipitate the drops on the vortex and place the test tubes in a magnetic rack for 1.5-2 minutes.[10]

2.13. Transfer supernatant with RNA to clean tubes and use for RT-PCR reaction.

After the work is completed, return the test tubes with NC and ICS to the freezer with temperature no higher than minus 20 °C.

When using Versions 1 and 3 of «CoV-2-Test» kit that do not contain the «CoV-2-Test-extraction» RNA extraction kit, the following extraction kits are recommended:

[8] If the sample volume is less than 100 μl, bring it to 100 μl using a sterile saline solution.

[9] In the absence of a magnetic rack, magnetic particles can be deposited by centrifugation at 10,000 g for 30 seconds

- Reagent kit for nucleic acids isolation (PROBA-NA/PROBA-NA-PLUS) according to TS 9398-035-46482062-2009, produced by DNA-Technology, LLC (Russia) (Registration Certificate No. FSR 2010/08867 dd. 13.10.2016),

During RNA isolation it is required to strictly observe the Protocol and Instructions of the used kits.

Conditions for possible storage of tested RNA samples

- at temperature of 2 to 8 °C no more than 4 hours (recommended),
- at temperature from minus 24 to minus 16 °C for no more than a week,
- at temperature no higher than minus 68 °C for no more than a year.

9.2. RT-PCR Process

Preparing RT-PCR kit components

1. Thoroughly mix the content of tubes with RNA extracted for testing, NC, RT-PCR buffer, primer mix, and PC, inverting each tube 10 times or mixing it on the vortex at a low speed for 3-5 seconds, and then precipitate the drops from the test tube caps by short centrifugation.

2. Select the required number of 0.1-0.2 ml tubes for RT-PCR reaction based on the number of tested RNA, PC and NC samples.

Before preparing the reactions, it is necessary to do wet cleaning of the PCR cabinet, including equipment and materials in it with the use of disinfectants suitable for use in PCR laboratories, turn on a UV lamp for 20-30 minutes.

Total volume of the reaction is 25 µl.

ATTENTION! It is not allowed to change the volume of the reaction. When the volume changes, the sensitivity of the method decreases dramatically!

To perform a single reaction, it is required:

When using RT-PCR reagent kit with 5x buffer «CoV-2-Test-RT-PCR-r» (Versions 1 and 2):

1. RT-PCR -buffer-r – 5 µl,
2. Primer-mix – 5 µl,
3. Sample (RNA, PC, NC testing sample after RNA exstraction stage) – 15 µl.

RT-PCR Protocol

Prepare reaction tubes according to Table 6 in the following order:

1. Label 0.1-0.2 ml tubes for RT-PCR, one for each test sample[10] , one tube for PC and one tube for NC after RNA isolation stage (Table 6).

Table 6 – Example of test tubes placement and components adding for PCR

Component	Sample 1		Sample 2		Controls	
	repeats		repeats		PC	NC
RT-PCR-buffer, µl	5	5	5	5	5	5
Primer-mix, µl	5	5	5	5	5	5
RNA sample, µl	15	15	15	15	-	-
PC, µl	-	-	-	-	15	-
NC, µl	-	-	-	-	-	15

2. Add 5 µl of RT-PCR buffer to each tube[11].

3. Add 1 µl reverse transcriptase to each tube[12].

4. Add 5 µl of primer-mix to each tube.

5. Add 15 µl of isolated RNA to each appropriate test tube[10]. It is necessary to make sure that before RNA isolation ICS has been added to biomaterial. RNA is not added to the PC and NC test tubes.

6. Add 15 µl of PC to the corresponding tube.

7. Add 15 µl of NC after RNA isolation stage to a corresponding test tube.

8. Precipitate drops from the walls, center the test tubes for 1-3 seconds on a vortex micro-centrifuge.

9. Place test tubes in reaction module of PCR device in “real time”. It is recommended to place the test tubes in the thermoblock center to evenly press the test tubes with the heating cover.

10. Program the device to perform the appropriate fluorescence signal amplification and detection, following the device use instructions. RT-PCR Protocol is given in Table 7.

Table 7 – RT-PCR Protocol for RT-PCR reagent kit with 5x buffer «CoV-2-Test-RT-PCR-r»

Stage	**Temperature, ˚C**	**Time, min: sec.**	**Detection Channels**	**Total cycles**
1	52	40:00		1
2	95	02:00		1

[10] To improve accuracy it is recommended to test each sample in two replicates

3	95	00:15		50
	64	00:20	FAM, HEX	

10. State the number of samples and their IDs, mark test tube location on the thermoblock matrix in accordance with their placement.

11. Make sure that FAM and HEX detection channels are used in the optical measurement parameters of the amplification program.

12. Start RT-PCR with fluorescent signal detection.

13. When the program is finished, start analyzing the results.

10. Registration and Interpretation of Results

Registration of results is performed automatically upon completion of RT-PCR using the software of a device used. The results are interpreted using the values of Ct ICS (HEX channel) and Ct of the coronavirus target (FAM channel).

First, a reaction rate and Ct values in control samples are assessed. Interpretation of the results in the testing samples starts only after correct results of PC and NC.

Interpretation of Results in Control Samples

For negative and positive control samples, the following results should be obtained (Table 8).

Table 8 - Testing results for negative and positive control samples

Material added	Selected fluorophore	
	FAM (coronovirus)	HEX (ICS)
NC	Ct >45 or absent	Ct >45 or absent
PC	Ct ≤35	Ct ≤35

If a negative control sample receives values that differ from those shown in Table 9, the results of the entire reactions are considered to be false. In this case, special measures should be taken to eliminate possible contamination.

If a positive control sample has values that differ from those indicated in Table 8, all samples must be amplified again.

Interpretation of Results in Testing Samples

The method for result interpretation is shown in Table 9 and is performed in the following order:

1. Determine the quality of isolated RNA for testing by Ct value for HEX channel, it should not exceed 35.

2. Evaluate the reaction result via FAM channel.

Table 9 – Result Interpretation Methodology

Threshold cycle value (Ct) by channels		Result
FAM	HEX	
absent	Ct ≤35	SARS-CoV-2 coronavirus RNA **not detected**
Ct ≤40	not count[11]	SARS-CoV-2 coronavirus RNA **detected**
Ct >40 or absent	Ct>35 or absent	Invalid result
Ct >40	Ct ≤35	Doubtful result

Result interpretation methodology is the following:

RNA of the SARS-CoV-2 coronavirus is not detected «-» if Ct on HEX channel is no more than 35, and Ct on FAM channel is not shown (no fluorescence accumulation graph).

RNA of the SARS-CoV-2 coronavirus is detected «+» if Ct on FAM channel is no more than 40. Result on HEX channel is ignored.

Invalid test result «?», if Ct on FAM channel is not specified (no fluorescence accumulation graph) or more than 40, and Ct on HEX channel is not specified (no fluorescence accumulation graph) or more than 35.

The reason for obtaining invalid results may be low RNA concentration, presence of inhibitors in RNA preparation obtained from clinical material, incorrect execution of testing protocol, non-compliance with RT-PCR temperature mode, etc.

The test result is doubtful if Ct on FAM channel is more than 40, and Ct on HEX channel is not more than 35.

In case of invalid and doubtful results, the report is not issued, it is necessary to re-collect a patient's biomaterial and repeat the test.

If a doubtful result is repeated, the sample is considered positive.

[11] At high concentrations of SARS-CoV-2 coronavirus RNA, HEX channel output may occur at late cycles or not occur.

11. Information about Stability of a Medical Product

Version	Expiration Date
Version 1	12 months
Version 2	12 months
Version 3	12 months
Version 4	12 months

Version	Expiration Date After Opening
Version 1	12 months
Version 2	12 months
Version 3	12 months
Version 4	12 months

12. Storage, transporting, use conditions

The kits «CoV-2-Test-RT-PCR-r» included in the composition of the kit «CoV-2-Test» should be stored at temperature not higher than minus 20 °C in the manufacturer's packaging for the entire shelf life.

After opening, store conditions are the same as before opening.

«CoV-2-Test-extraction» included in Versions 2 should be stored at temperature of 2 °C to 25 °C in the manufacturer's packaging in a place protected from light for the entire shelf life. Freezing of reagents is not allowed.

After opening, store conditions are the same as before opening.

A kit stored in violation of storage conditions cannot be used.

Transporting

«CoV-2-Test» kits must be transported by all types of covered vehicles in accordance with the transport rules applicable to this type of transport.

«CoV-2-Test-RT-PCR-r» included in the kit «CoV-2-Test» should be transported at a temperature of minus 20 °C during the entire shelf life of a kit. It is allowed to transport at a temperature of 4 °C for up to 10 days, at room temperature (15-25 °C) for no more than two days.

«CoV-2-Test-extraction» kit included in Versions 2 should be transported at a temperature of 2 to 25 °C. Freezing of reagents is not allowed.

Atmospheric pressure is not controlled, because it does not affect the quality of the product.

To ensure compliance with transportation conditions throughout the entire transportation period, a reagent kit is placed in a reusable polyurethane foam thermal container with ice pack for temporary storage and transportation with prepared refrigerating elements. The type, volume and number of icepacks put in the cold box of thermal container with reagent kits, and the thermal container size varies according to the duration and conditions of transportation.

Reagent kits transported with violation of temperature conditions are not to be used.

Shelf Life

The shelf life of the kit «CoV-2-Test» in all versions is 12 months from the date of acceptance by the manufacturer's Quality Control Department subject to compliance with all transportation, storage and use conditions. A reagent kit with an expired expiration date are not to be used.

Shelf life of opened kit components

12 months from the date of acceptance by the manufacturer's Quality Control Department, provided that the kit «CoV-2-Test-RT-PCR-r» are stored at a temperature of minus 20 °C; «CoV-2-Test-extraction» (Version 2) – at a temperature of 2°C to 25°C.

Shelf life of kit components ready for use

1 hour under conditions that prevent drying of components, their contamination with foreign biological material.

The mixture prepared from the lysis buffer, additional component for lysis and magnetic microbeads must be stored at a temperature of 2 to 8°C for a month.

13. Utilization

Kits that have become unusable including because of the expiration of the expiry date, are subject to utilization in accordance with the requirements of SanPiN 2.1.7.2790-10 «Sanitary and epidemiologic requirements to the address with medical waste».

In accordance with classification of medical waste the kits refer to class A (epidemiologically safe waste approached on structure to municipal solid waste). Unused reagents in accordance with p. 4.28 of SanPiN 2.1.7.2790-10 «Sanitary and epidemiologic requirements to the address with medical waste» are not subject to use gather in the one-time marked packaging of any color (except yellow and red).

Residual tubes and materials are utilized in accordance with MU 287-113 (Methodological instructions for disinfection, pre-sterilization purification and sterilization of medical devices).

Liquid components are eliminated by draining into the sewage system with a preliminary watering of the reagent with tap water 1: 100 and removal of the rest of the packages as industrial or household garbage.

Consumer package of «Test-NRAS-tissue» kit is subject to mechanical destruction with removal of residues as industrial or household garbage.

Personnel carrying out the destruction of the kit must comply with the safety rules for carrying out a particular method of destruction.

14. Warranty Obligations, Contacts

The manufacturer guarantees the conformity of «CoV-2-Test» kit to technical requirements under transportation, storage and operation conditions established by technical specification.

If there are any complaints regarding the quality, undesired events that may cause adverse event (incident), send the information to the address:

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