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INSTRUCTION FOR USE

CoV-Influ-Test Reagent Kit for Detecting SARS-CoV-2 RNA, Influenza A Virus and Influenza B Virus by Real-Time RT-PCR

TU 21.20.23-026-97638376-2020

Version 2 of 22.07.2021

Table of Contents

Introduction

Diagnosis of SARS-CoV-2 coronavirus, Influenza A virus and Influenza B virus is extremely important for correct and timely diagnosis and treatment of SARS symptoms.

The target analytes: specific parts of the genomic RNA of the SARS-CoV-2 coronavirus infection, of Influenza A virus (IAV) and Influenza B virus (IBV).

The scientific validity of the target analyte lies in its specificity (uniqueness of RNA sequence) in relation to the genomes of detected viruses.

Highly conserved fragments of the *N* and *RdRp* genes (open reading frame 1ab, *ORF1ab*) are used as a target for detecting the RNA of the SARS-CoV-2 virus. Simultaneous detection of two targets reduces the probability of obtaining false negative results due to the high molecular genetic polymorphism of SARS-CoV-2 (including lines B.1.1.7, B.1.617, B.1.351, P.1 and P.2 (www.gisaid.org)). The target for detecting the RNA of the influenza A virus is a fragment of the *M1* gene (matrix gene)[1]. The target used allows detecting all known virus strains, including H1N1, H3N2, H5N1, H7N9, H9N2, H10N8, but without differentiating them. The target for detecting the RNA of the influenza B virus is a fragment of the *NP* gene (nucleoprotein gene).[1]

The use area of the reagent kit is clinical laboratory diagnostics of infectious diseases.

Indications and Contraindications for Use

Indications for use: CoV-Influ-Test reagent kit is recommended for use in patients with symptoms of respiratory disease suspected of infection caused by SARS-CoV-2, Influenza A virus, IAV or Influenza B virus, IBV to clarify the etiology of the disease and the choice of appropriate antiviral therapy, prognostic assessment of the course of the disease and possible complications.

[1] WHO information for the molecular detection of influenza viruses. July 2017. P. 1–60.

Contraindications for use: No contraindications have been identified when the medical device is used by specially trained personnel and based on its intended use.

Population, demographic aspects of the use of the medical device: no population, demographic aspects of the use of the CoV-Influ-Test reagent kit have been identified.

Sterility: the product is not sterile.

1. Intended Use

Intended Use: the CoV-Influ-Test reagent kit is designed for qualitative detection of specific parts of the genomic RNA of the SARS-CoV-2 coronavirus infection, of Influenza A virus (IAV) and Influenza B virus (IBV) by the real-time single-stage reverse-transcription – multiplex allele-specific polymerase chain reaction with hybridization-fluorescent detection (real-time RT-PCR) in the RNA specimen extracted from human clinical material (nasopharynx swabs, oropharyngeal swabs, sputum) in patients suspected to be infected by coronavirus infection SARS-CoV-2, Influenza A virus (IAV) or Influenza B virus (IBV) to clarify the etiology of the disease and the choice of appropriate antiviral therapy, prognostic assessment of the course of the disease and possible complications.

Functional purpose: The obtained results can be used to diagnose respiratory diseases caused by the SARS-CoV-2 virus, Influenza A virus and Influenza B virus.

Potential users of the medical device

The kit is designed for the professional use in medical institutions and in clinical diagnostic laboratories. Professional level of potential consumers – a doctor of clinical laboratory diagnostics, medical laboratory technician.

In accordance with clause 3.4. of Methodological Recommendations MR 3.1.0170-20 “Epidemiology and Prevention of COVID-19”: “Primary screening assays without extraction can be carried out on the basis of laboratories that have Hygiene Certificate for working with pathogens of infectious diseases of groups III-IV pathogenicity. At that the screening can be carried out by personnel who have given their written consent and have been trained/instructed on ensuring biological safety requirements by employees of the organization of Rospotrebnadzor allowed to work with pathogens of infectious diseases

of group II of pathogenicity (such trainings are organized at the territorial level by collecting applications and organizing training groups with the approval by the territorial bodies of Rospotrebnadzor)".

2. Method Principle

Method

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

Test sample type

The material for the study is RNA specimens extracted from human clinical samples: nasopharynx swabs, oropharyngeal swabs, sputum.

Collection of biological material, sample preparation, storage, its subsequent neutralization and disposal should be carried out following the Instruction for use for the reagent kit, in accordance with instructional and methodological documents regulating performance of tests for the infectious agent of COVID-19 infection, and Health Regulations 1.3.3118-13 ("Safety When Working with Microorganisms in Pathogenicity (Hazard) Groups I and II", Provision of November 28, 2013 No. 64 and Temporary Guidelines "Prevention, Diagnosis and Treatment of Novel Coronavirus Infection (COVID-19)".

All samples collected for laboratory testing should be considered potentially infected, and when working with them, the requirements of Health Regulations 1.3.3118-13 "Safety When Working with Microorganisms in Pathogenicity (Hazard) Groups I and II" should be adhered. Health providers who collect or transport clinical samples to the laboratory should be trained to safe handle biomaterials, strictly observe precautionary measures and use personal protective equipment (PPE).

Detection Principle

Qualitative identification of specific regions of the genomic RNA of the SARS-CoV-2 virus, Influenza A virus and Influenza B virus by a multiplex single-stage reverse transcription reaction followed by an allele-specific polymerase chain reaction with real-time hybridization-fluorescence detection (RT-PCR in real-time) in an RNA specimen extracted from clinical specimens includes three stages:

1. Preparation for RT-PCR;

2. Reverse-transcription polymerase chain reaction (RT-PCR) of RNA producing complementary DNA (cDNA) which can then be amplified and detected with real-time hybridization-fluorescence detection;

3. Interpretation of results.

Single-stage reverse transcription reactions and amplification of specific regions are carried out with RNA specimens using primers specific to them in the reaction buffer.

The RT-PCR buffer includes all the main reagents, including a "warm start" reverse transcriptase, "hot start" thermostable DNA polymerase, deoxynucleotide triphosphates and an optimized buffer.

The oligonucleotide mixture contains primers and fluorescently labeled oligonucleotide probes that hybridize with an amplified complementary target DNA and are hydrolyzed (destroyed) by *Taq*-polymerase, as a result of which the dye and quencher are separated, and the fluorescence intensity increases over the corresponding range of the optical spectrum. This makes it possible to register the accumulation of a specific amplification product by measuring the intensity of the fluorescent signal in real time.

The kit contains reagents for multiplex detection of highly specific regions (targets) of genomic RNA of SARS-CoV-2 viruses, Influenza A Virus and Influenza B Virus, and Internal Control (IC) (Table 1).

Table 1 – Assay Targets

Channel Corresponding to Fluorophore			
FAM / Green	**HEX / Yellow**	**ROX / Orange**	**Cy5 / Red**
RNA of SARS-CoV-2 (the *N* and *RdRp* gene fragments)	IC	RNA of Influenza A Virus (*M1* gene fragment)	RNA of Influenza B Virus (*NP* gene fragment)

IC allows evaluating the quality and efficiency of RNA extraction and determining the presence of possible reverse transcription and amplification inhibitors in a specimen, the presence of which can lead to false negative results.

Method Limitation

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

Breach of the package integrity during transportation.

The use of an expired kit or the kit that was stored in inappropriate conditions.

Violation of storage conditions and transporting conditions of samples.

The time of RT-PCR reaction is from 100 to 125 minutes (excluding time for sample preparation) depending on the used amplifier.

3. Reagent Kit Components

The CoV-Influ-Test reagent kit is produced in one design version.

Number of Tests

The reagent kit (Table 2) is designed to conduct 96 reactions. That means that in a single start of the amplifier for 96 wells it is possible to study 94 test samples and a negative control and positive control or to perform 32 single setups of test samples with a negative control and positive control.

Table 2 – Components of CoV-Influ-Test Reagent Kit

No.	Reagent	Description	Quantity, Volume
1.	RT-PCR-buffer 5x	Transparent colorless liquid	1 test tube (480 µl)
2.	Oligonucleotide mixture	Transparent liquid of lilac color,	1 test tube (480 µl)
3.	PC	Transparent colorless liquid	1 test tube (480 µl)
4.	NC	Transparent colorless liquid	2 test tubes (1 600 µl each)
5.	ICS	Transparent colorless liquid	1 test tube, 950 µl

Kit Components

The **RT-PCR buffer 5x** includes all the main reagents, including a "warm start" reverse transcriptase, "hot start" thermostable DNA polymerase, deoxynucleotide triphosphates and an optimized buffer.

The mixture of oligonucleotides is ready for use and contains primers and probes designed to detect specific targets (Table 1).

Positive Control (PC) is ready for use and it is a mixture of plasmid DNA with synthetic inserts of amplified DNA fragments: specific cDNA fragments of the *N* and *RdRp* genes of the novel coronavirus infection viruses, the *M1* gene of the Influenza A Virus and the *NP* gene of the Influenza B Virus and IC.

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

Internal Control (IC) is a reinforced nucleic acid (NA) specimen, ready for use.

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

4. Reagent Kit Characteristics

4.1. Technical and Functional Characteristics

Table 3 – CoV-Influ-Test Reagent Kit

Parameter	Characteristics and Standards	Clause in Technical Specification (TU)
1. Technical Characteristics		**1**
1. Visual appearance		
RT-PCR-buffer 5x	Transparent colorless liquid	Section 7, Clause 7.6
Oligonucleotide mixture	Transparent liquid of lilac color,	Section 7, Clause 7.6
Positive Control (PC)	Transparent colorless liquid	Section 7, Clause 7.6
Negative Control (NC)	Transparent colorless liquid	Section 7, Clause 7.6
Internal Control (IC)	Transparent colorless liquid	Section 7, Clause 7.6

1.2. Kit Components	In accordance with p.1.4 of TU 21.20.23-026-97638376-2020	Section 7, Clause 7.12
Labelling	In accordance with p.4 of TU. 21.20.23-026-97638376-2020	Section 7, Clause 7.12
Packaging	In accordance with p. 5 of TU 21.20.23-026-97638376-2020	Section 7, Clause 7.12
2. Functional Characteristics		
2.1 Positive result with PC	Detection of the increase of fluorescence signal in the test tubes with PC via FAM, HEX, ROX and Cy5 channels, Ct≤30	Section 7, Clause 7.8.2
2.2 Negative result with NC	In the test tubes with NC via FAM, HEX, ROX and Cy5 channels, Ct is not shown (that is, there is no graph of fluorescence accumulation)	Section 7, Clause 7.8.2
2.3 Reaction in test tubes with Specificity Control (SC)	In the test tubes with SC, Ct is not shown via FAM, ROX and Cy5 channels (that is, there is no graph of fluorescence accumulation) and via HEX channel Ct≤32.	Section 7, Clause 7.8.2
2.4 Reaction in test tubes with Sensitivity Control (SenC)	In the test tubes with SenC-1 via FAM and HEX channels in all repetitions (at least 3) Ct≤35	Section 7, Clause 7.8.2
	In the test tubes with SenC-2 via FAM and HEX channels in all repetitions (at least 3) Ct≤35.	
	In the test tubes with SenC-3 via HEX and ROX channels in all repetitions (at least 3) Ct≤35	
	In the test tubes with SenC-4 via HEX and Cy5 channels in all repetitions (at least 3) Ct≤35	

4.2. Analytical Performance Characteristics

Analytical specificity	It is specific to RNA fragments of SARS-CoV-2 viruses and Influenza A Virus and Influenza B Virus and to Internal Control. The absence of non-specific positive amplification results was shown in the presence of the following organisms and viruses in the genomic RNA sample: Human (in a

	concentration of up to 109 copies/ml of the sample), Human coronavirus 229E, Human coronavirus OC43, Human coronavirus HKU1, Human coronavirus NL63, SARS-coronavirus, MERS-coronavirus, Adenovirus, Human metapneumovirus, Parainfluenza virus 1-4, Enterovirus, Respiratory syncytial virus, Rhinovirus, Cytomegalovirus, Epstein Barr virus, Human alphaherpesvirus 1, Human alphaherpesvirus 2, Human herpesvirus 6, Human herpesvirus 8, *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. canettii*, *M. caprae*, *M. microti*, *M. avium*, *M. abscessus*, *M. septicum*, *M. fortuitum*, *M. gordonae*, *M. intracellulare*, *M. kansasii*, *M. marinum*, *M. smegmatis*, *M. xenopi*, *M. ulcerans*, *M. terrae*, *Mycolicibacterium* spp., *Mycobacteroides* spp., *Gordonia* spp., *Tsukamurella* spp., *Nocardia* spp., *Corynebacterium* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *St. epidermis*, *St. salivarius*, *Bordetella* sp., *Haemophilus influenzae*, *Chlamydophila pneumoniae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Corynebacterium diphtheriae*, *Candida albicans*
Analytical sensitivity	At least 1,000 copies of RNA of SARS-CoV-2 viruses, Influenza A Virus and Influenza B Virus per 1 ml of a clinical specimen when extracted from 100 µl and elution with a volume of 50 µl, for other volumes the sensitivity value changes proportionally. With an increase in the volume of a clinical specimen and a decrease in the volume of elution, a proportional increase in sensitivity occurs.

4.3. Clinical Efficiency

Table 4 – Clinical Efficiency

Tested material	**Number of tests**	**Diagnostic sensitivity**	**Diagnostic specificity**	**Confidence Interval** with 95 % of Confidence probability
Oropharyngeal swabs	45	100%	100%	100% (95% CI:96%-100%)
Nasopharynx swabs	32	100%	100%	100% (95% CI:96%-100%)
Sputum	10	100%	100%	100% (95% CI:96%-100%)

5. Risks Associated With the Use of the Reagent Kit

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 clinical specimens with inhibitory substances in concentrations exceeding the permissible;
3. Contamination of reaction mixtures and RNA specimens with the PC test tube content or with amplification products;
4. The test is performed using a low-quality RNA specimen (low concentration and/or poor purification);
5. Failure to meet the requirements for sample preparation, testing and disposal due to unskilled personnel work with the kit;
6. Use of an 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 device “CoV-Influ-Test Reagent Kit for Detecting SARS-CoV-2 RNA, Influenza A Virus and Influenza B Virus by Real-Time RT-PCR” is acceptable, the benefit of its use exceeds the risk.

6. Precautions When Working With the Kit

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 samples and materials during testing should be handled in accordance with laboratory practice for infectious diseases, and in

accordance with clause 4.2.3 of Temporary Guidelines "Prevention, Diagnosis and Treatment of the Novel Coronavirus Infection COVID-19":

All samples collected for laboratory testing should be considered potentially infected, and when working with them, the requirements of Health Regulations 1.3.3118-13 "Safety When Working with Microorganisms in Pathogenicity (Hazard) Groups I and II" should be adhered. Health providers who collect or transport clinical samples to the laboratory should be trained to safe handle biomaterials, strictly observe precautionary measures and use personal protective equipment (PPE).

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 tested samples, premises and equipment with nucleic acids and (or) amplicons.

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 2.1.3684-21 "Sanitary and epidemiological requirements for maintenance of territories of urban and rural settlements, for water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures". Personnel should follow recommendations set out in Methodology Guidelines 287-113 "Disinfection, Pre-Sterilization Cleaning and Sterilization of Medical Devices", Methodology Guidelines 1.3.2569-09 "Organization of Work in Laboratories Using Methods of Nucleic Acid Amplification When Working With Material Containing Microorganisms of Pathogenicity Groups I-IV".

When working it is required:

- remove unused reagents in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for maintenance of territories of urban and rural settlements, for water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures".

ATTENTION! When removing waste after amplification (test-tubes containing 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 I and II 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.

All components and reagents included in the CoV-Influ-Test reagent kit 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 IC reagent contains sodium azide in a concentration not exceeding 0.1 %. This reagent is not classified as dangerous, does not require special precautions and is not subject to special labeling in accordance with GOST 31340-2013.

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

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

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. Required Equipment and Materials

The reagent kit is operated in working area 3 (for preparation of reactions) (Methodology Guidelines 1.3.2569-09).

Equipment Required for Multiplex RT-PCR Reaction:

1. Biosafety cabinet, biological safety Class II and III;
2. Vortex;
3. Set of electronic or automatic variable volume dispensers;
4. Refrigerator for +2 °C to +8 °C with a freezer not higher -16 °C;
5. Thermal cycler (PCR machine)[2] with real-time fluorescence detection via channels corresponding to FAM/Green and HEX/Yellow, e.g., CFX96 (BioRad, USA), DTprime (DNA-Technology, Russia), Rotor-Gene Q (Qiagen, Germany), QuantStudio 5 (Thermo Fisher Scientific, USA), Gentier 96 (Tianlong, China).

Materials and Reagents Required But Not Provided:

ATTENTION! It is required to use only disposable sterile plastic consumables that have a special "DNase-free" and "RNase-free" marking.

1. Disposal pipette tips with an aerosol barrier up to 1,000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA);
2. Disposal sterile Eppendorf type test tubes, 1.5 or 2.0 µl;
3. Thin-wall disposable PCR tubes, an optically transparent cap (in the case of detection through the cap) or optically transparent walls (in the case of detection through the tube wall): 0.1 or 0.2 ml PCR tubes, or 0.1 or 0.2 ml PCR tubes in strips, or PCR plates with an optically transparent film (e.g., Axygen, USA), compatible with the used thermal cycler;
4. Isolation gown coat and disposable talc-free gloves;
5. Container with disinfectant;
6. "Workplace" racks for 0.1 ml or 0.2 ml test tubes or for 0.1 ml or 0.2 ml tube strips;
7. The kit for RNA extraction from clinical specimens (see clause 8.2).

[2] Thermal cyclers should 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.

8. Test Samples

Test sample type

Material for the study is RNA specimens extracted from human clinical specimens: oropharyngeal swabs, nasopharynx swabs, sputum.

8.1. Clinical Material Preparation

ATTENTION! Before starting the 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**.**

Clinical specimens are 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 II.

Collection of Specimens for Testing

Nasopharynx and Oropharyngeal Swab

Oropharyngeal Swab. Swabs are collected with dry sterile cotton swabs with plastic shafts by rotating the swab on tonsils, faucial pillars, and on the back of the throat.

Nasopharynx Swab. Swabs are collected with dry sterile cotton swabs with plastic shafts. A swab is gently inserted through the nostril along its outer wall to a depth of 2-3 cm to the inferior nasal concha. Then it is slightly lowered down, inserted into the lower nasal passage under the lower nasal concha, rotated and removed along the outer wall of the nostril.

After that a swab with collected specimen on a soft end is placed into a disposable sterile Eppendorf type test tube containing transport medium (or 500ml sterile saline solution), then a plastic shaft is carefully broken off at a distance of no more than 0.5 cm from the cotton swab, the soft end (cotton swab) of the probe is left with the specimen inside the test tube. 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. Specimens are collected in 50 ml disposable graduated sterile containers with wide screw cap.

Clinical Material Storage and Transportation Conditions:

Sample	Material collection requirements	Transportation	Storage conditions prior testing	Comments
Nasopharynx and oropharyngeal swabs	Plastic test tubes and sample collection swabs [3]	4°C	<= 5 days: 4°C > 5 days[4]: -70°C	Nasal and oropharyngeal swabs should be placed in one and the same test tube to increase viral load
Sputum	Sterile container[5]	4°C	<= 48 hours: 4°C > 48 hours: -70°C	Make sure that the material is coming from the lower respiratory tract

Only a single freezing-thawing of the material is allowed.

Material Preliminary Preparation

Collection of biological material, sample preparation, storage, its subsequent neutralization and disposal should be carried out following the instruction for use for the reagent kit, in accordance with instructional and methodological documents regulating performance of tests for the infectious agent of COVID-19 infection, and Health Regulations 1.3.3118-13 ("Safety When Working with Microorganisms in Pathogenicity (Hazard) Groups I and II", Provision of November 28, 2013 No. 64 and Temporary Guidelines "Prevention, Diagnosis and Treatment of Novel Coronavirus Infection (COVID-19)".

All samples collected for laboratory testing should be considered potentially infected, and when working with them, the requirements of Health Regulations 1.3.3118-13 "Safety When Working with Microorganisms in Pathogenicity (Hazard) Groups I and II" should be adhered. Health providers who collect or transport clinical samples to the laboratory should be trained to safe handle biomaterials, strictly observe precautionary measures and use personal protective equipment (PPE).

8.2 RNA Extraction from Clinical Specimens

To extract RNA from a human clinical specimen (nasopharynx swabs, oropharyngeal swabs, sputum), it is recommended to use the following reagent kits:

[3] For the transportation of samples, transport medium containing antifungal and antibiotic additives is used.

[4] If it is not possible to store samples at minus 70°C, store them at minus 20°C.

- NA extraction reagent kit (PREP-NA/PREP-NA-PLUS), TU 9398-035-46482062-2009, DNA-Technology LLC, Russia (Registration Certificate No. FSR 2010/08867 of 13.10.2016);

or similar designed for DNA extraction from blood plasma and providing the following quality of extracted DNA

- purity of extracted DNA/RNA, A_{260}/A_{280}, not less 1.7;
- DNA/RNA extraction efficiency – at least 25%.

During RNA extraction procedure, strictly follow the protocol and the manufacturer's instructions of the reagent kit used.

Add 10 μl of IC from the CoV-Influ-Test reagent kit to each specimen.

RNA is also extracted from NC specimen in the volume of 100 μl, 10 μl of IC is added. If the manufacturer's instruction for RNA extraction recommends to use a bigger amount of specimen, NC volume should be increased with adding saline solution or TE-buffer.

Possible Storage Conditions for RNA Specimens:

- at a temperature of 2 to 8°C maximum 4 hours (recommended),
- at a temperature of minus 24 to minus 16°C maximum one week,
- at a temperature not higher minus 68°C maximum one year.

8.3. Interfering Substances and Restrictions on the Use of Testing Specimens

The effect of potentially interfering substances on the CoV-Influ-Test reagent kit was tested for potentially interfering substances that will occur during the clinical material sampling in the following concentration:

- Hemoglobin – 1 %;
- Mucin – 2 %;
- Phenylephrine – 15% v/v
- Sodium chloride – 5% v/v
- Cromolyn – 15% v/v
- Oxymetazoline – 15% v/v
- Fluconazole – 5% m/v
- Benzocaine, Menthol – 0,15% m/v
- Galphimia Glauca, Sabadilla – 20% v/v
- Zinc Gluconate – 5% m/v
- Alkalol – 10% v/v

- Fluticasone Propionate – 5% v/v
- Phenol – 15% v/v
- Tamiflu (oseltamivir phosphate)– 0,5% v/v
- Mupirocin – 0,25% v/v
- Tobramycin – 0,0004% m/v

Based on the study results, potentially interfering substances encountered during RNA extraction from clinical specimens evaluated at concentrations that are expected to occur with normal use of the CoV-Influ-Test reagent kit do not have an interfering effect on the test result.

Restrictions on the Use of Testing Specimens:

- testing material is not subject to use in case of violation of storage and transportation conditions (temperature, duration, repeated freezing and thawing);
- use of specimens contaminated with foreign biological material is not allowed.

9. Preparation of Kit Components for Testing

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

ATTENTION! When working with RNA, it is required to use only disposable sterile plastic consumables that have a special marking “DNase-free” and “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 2 immediately before the testing.

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.

1. Thoroughly mix the content of test tubes with extracted RNA, RT-PCR buffer, oligonucleotides, NC and PC, upturning each test-tube 10 times or vortex at low speed for 3-5 sec., then remove the drops from the test tube caps by short centrifugation.

2. Select the required number of 0.1 ml or 0.2 ml PCR test tubes (with optically transparent caps or optically transparent walls - depending on the type of detection) following the calculation: 1 x number of specimens + 1 x PC + 1 x NC.

10. Testing Procedure

PCR testing consists of the following stages:

1. Preparation of RT-PCR;
2. Reverse transcription of RNA and PCR amplification of cDNA with hybridization-fluorescence detection of amplification products in real time;
3. Interpretation of results.

A) Preparation of RT-PCR

(conducted in pre-PCR ZONE, in the room for pipetting reagents and preparation for PCR-amplification).

Total volume of the reaction is 25 µl.

ATTENTION! It is not allowed to change the volume of the reaction. To prepare reaction mixture it is required to use:

1. RT-PCR-buffer 5x – 5 µl,
2. Oligonucleotides – 5 µl,
3. Specimen (RNA specimen, PC, NC) – 15 µl.

Prepare reaction tubes according to Table 2 in the following order:

1. Label 0.1-0.2 ml tubes for PCR.

2. In a separate disposable sterile Eppendorf type test tube, 1.5 or 2.0 ml, prepare a reaction mixture: (n+3)x5 µl of PCR buffer and (n+3) x5 µl of oligonucleotides, where n is the number of specimens. Thoroughly vortex the reaction mixture for 3-5 seconds.

3. Add 10 µl of the reaction mixture to the test tubes prepared for PCR.

4. Add 15 µl of extracted RNA to each corresponding test tubes for specimens. Do not add RNA specimen to PC and NC test tubes.

5. Add 15 µl of PC to the corresponding test tube.

6. To the corresponding test tube add 15 µl of NC, after NA extraction stage.

7. Centrifuge the test tubes for 1-3 seconds to remove the drops from the walls, use a micro-centrifuge vortex.

B) Reverse transcription of RNA and PCR amplification of cDNA with hybridization-fluorescence detection of amplification products in real time;

(conducted in the PCR ZONE, in the room for PCR-amplification)

1. Place the test tubes in the reaction module of the real-time PCR machine. It is recommended to place the test tubes in the center of the thermal cycler for uniform pressing of the test tubes with a heating lid.
2. Program the device to perform the correspondent RT-PCR program and fluorescent signal detection, following the instruction for use. RT-PCR Protocol is shown in Table 5.

ATTENTION! In the case of using QuantStudio 5, configure the optical filters before starting the amplification protocol (it is possible to register the VIC signal with the ROX channel in the case of ΔRn > 100, 000, which can lead to false positive results)! To do this, click the "Action" button in the "Method" tab, then select "Optical filter settings" in the pop-up menu, where in the "PCR Filter" section, leave only the following filter combinations: x1 – m1, x2 – m2, x4 – m4, x5 – m5, x6-m6.

Table 5 – RT-PCR Protocol

Stage	Temperature, °C	Time, min.: sec.	Detection Channels	Total Cycles
1	52	40:00	–	–
2	95	02:00	–	–
3	95	00:15	–	5
	64	00:20		
4	95	00:05	–	45
	64	00:30	FAM/Green, HEX/Yellow, ROX/Orange, Cy5/Red	

Specify the number and identifiers of specimens, mark the location of the tubes on the matrix of the thermal cycler in accordance with their placement.
3. Make sure that the detection channels FAM/Green, HEX/Yellow, ROX/Orange and Cy5/Red are used in the optical measurement parameters of the amplification program.
4. Start RT-PCR with fluorescent signal detection.
5. When the program is finished, start analyzing the results.

11. Registration and Interpretation of Results

Registration of results is performed automatically upon completion of RT-PCR using the software of a device used.

Recommendations for setting the threshold line

For PCR machines of any model, the threshold line is set individually for each channel at a level corresponding to 10-20% of the maximum fluorescence level obtained for a positive control sample in the last amplification cycle.

The results are interpreted by Ct values of FAM/Green, HEX/Yellow, ROX/Orange and Cy5/Red channels (Table 1). Only Ct values obtained at the PCR stage with fluorescence detection are taken into account (corresponding to stage 4 – see Table 5).

First, the reaction and Ct values in the control samples are evaluated. The interpretation of the results in the specimens begins only with the correct reaction with PC and NC.

ATTENTION! In the case of using the Rotor-Gene 6000, Rotor-Gene 3000, Rotor-Gene Q and similar PCR machines, activate the Dynamic Tube function, Noise slope correction, set the 10% value in the Outlier Removal section for all detection channels, except for Cy5, for which you need to set the 15% value in the Outlier Removal section.

Interpretation of Results in Control Samples

For negative and positive control samples, the following results should be obtained (Table 6).

Table 6 – Testing results for negative and positive control samples

Control	Ct values by detection channels corresponding to fluorophores			
	FAM / Green	HEX / Yellow	ROX / Orange	Cy5 / Red
NC	> 35 or absent	≤ 32	> 35 or absent	> 35 or absent
PC	≤30	≤32	≤30	≤30

If a negative control receives values that differ from those shown in Table 6, the results of the entire test are considered unreliable. In this case, special measures should be taken to eliminate possible contamination.

If a positive control has values that differ from those indicated in Table 6, repeated amplification of all the samples is required. When PC values differ from those indicated in Table 6 for the second time, it is necessary to change the reagents.

Interpretation of Results

The method for result interpretation is shown in Table 7.

If an increase in the fluorescence of a specific product is recorded for the specimen via the FAM/Green, ROX/Orange or Cy5/Red channels earlier than cycle 12 (Ct < 12), this indicates a high initial RNA concentration of the corresponding virus. In this case, it is possible to obtain a false negative result for a virus whose RNA is present in a low concentration. To exclude false negative results, it is recommended to perform RT-PCR/PCR with extracted RNA specimen or use reagent kits for separate detection RNA of the corresponding viruses.

Table 7 – Result Interpretation Methodology

Ct values for detection channels corresponding to fluorophore (target analytes are given in brackets)				Result
FAM / Green (SARS-CoV-2)	ROX / Orange (Influenza A Virus)	Cy5 / Red (Influenza B Virus)	HEX / Yellow (IC)	
–	–	–	≤ 32	RNA of SARS-CoV-2 Virus, Influenza A Virus and Influenza B Virus is not detected
–	–	–	> 32	invalid result
≤ 35			not count	Virus RNA is detected, corresponding to the Channel
> 35			not count	Test result is doubtful for the target corresponding to the channel

"Not count" means the result is not count at interpretation; "–" no fluorescent signal.

Invalid result may be obtained at low NA concentration, inhibitors presence in an NA sample obtained from clinical specimen, incorrect performance of test protocol, non-compliance with RT-PCR temperature conditions, etc.

Controversial result may be caused by a small concentration of the virus in the clinical sample.

In case of an invalid and doubtful result, the conclusion is not issued, it is necessary to re-collect biomaterial from the patient and re-conduct the test. And also, for doubtful results, it is recommended to extract NA from a larger volume of a clinical specimen. If a questionable result is repeated, repeat the test with a reagent kit from another manufacturer or using another method.

12. Storage, Transportation and Usage Conditions

Storage

CoV-Influ-Test reagent kit in the manufacturer' package should be stored at temperatures from minus 18 to minus 22 °C C during the total shelf life period. The storage at a temperature from 2 to 8 °C is possible for maximum 30 days.

Repeated (not more than 10 times) freeze-thaw cycles is allowed.

After opening the reagents are stored in the same conditions as packed reagents.

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

Transporting

The CoV-Influ-Test reagent kit can be transported by all types of covered vehicles in accordance with the transport rules applicable to this type of transport.

The CoV-Influ-Test reagent kit should be transported at a temperature from minus 18 to minus 22 °C during the total shelf life period. It is possible to transport the kit at a temperature from 2 to 8 °C during 30 days, or at a temperature from 15 to 25°C during not more than 5 days.

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 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.

A reagent kit transported in violation of temperature conditions cannot be used.

Shelf Life for the CoV-Influ-Test reagent kit is from the date of acceptance by the manufacturer's Quality Control Department, provided that all conditions of transportation, storage and operation are observed. A reagent kit with expired shelf life cannot be used.

Shelf Life of Opened Kit Components is 12 months from the date of acceptance by the manufacturer's Quality Control Department, provided that the kit components are stored at a temperature of minus 18 to minus 22 °C.

Expiration Time for the Kit Components Prepared for Use

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

13. Disposal

Reagent kits that have become unusable, including shelf life expiration, are subject to disposal in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for maintenance of territories of urban and rural settlements, for water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures".

According to medical waste classification, the kits belong to Class A (epidemiologically safe waste close in composition to solid household waste). Unused reagents in accordance with Section X, clause 170 of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for maintenance of territories of urban and rural settlements, for water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures" are collected in a single-use labeled packaging of any color (except yellow and red).

Test tubes and materials after the use are disposed in accordance with Methodology Guidelines 287-113 (Methodology Guidelines for Disinfection, Pre-Sterilization Cleaning and Sterilization of Medical Devices).

Liquid components (reagents) are disposed by draining into a sewer with preliminary dilution of a reagent with tap water 1: 100 and removing the remains of packages as industrial or household garbage.

Consumer packaging of the CoV-Influ-Test reagent kit is subject to mechanical destruction with the removal of residues as industrial or household garbage.

Personnel disposing reagents must comply with the safety rules for conducting a particular method of disposal.

14. Warranty Obligations, Contacts

The manufacturer guarantees quality and safety of the CoV-Influ-Test reagent kit during its shelf life subject to compliance with established requirements for transportation, storage and use.

In case of complaints about reagent kit quality, undesirable events or incidents, submit information to:

Limited Liability Company "TestGene"
(TestGene, LLC),
9 44th Inzhenerny Proezd, office 13, Ulyanovsk 432072
Tel.: +7 (499) 705-03-75
www.testgen.ru

Technical Support Service:
Tel.: +7 927 981 58 81
E-mail: help@testgen.ru

Annex B

Label Symbols

Symbol	Meaning
	Expiry date
	Manufacture date
	Lot number
	Catalogue number
	Consult instructions for use
	Temperature range
	The content is enough to be used for 94 detections
	*in vitro* diagnostic medical device