



INSTRUCTIONS FOR USE

**Reagent kit for qualitative detection of coronavirus RNA
(*SARS-CoV-2*)
by direct RT-PCR-RT "Cito-CoV-2-Test"**

TS 21.20.23-028-97638376-2020

Version 3 dated 24.03.2022

*Red-marked rules, standards etc. are local. They should be replaced by relevant ones applicable in a given country.

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List of abbreviations

The following abbreviations and designations are used in this instruction:

PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
NC	negative control sample
PC	positive control sample
ICS	internal control sample
SC	specificity control sample
SenC	sensitivity control sample

Introduction

Target analyte: a specific region of SARS-CoV-2 (2019-nCoV, COVID-19) strain genomic RNA, a fragment of *N* gene encoding the nucleocapsid phosphoprotein.

The scientific validity of the target analyte lies in its specificity (RNA/DNA sequence uniqueness) in relation to SARS-CoV-2 (2019-nCoV, COVID-19) strain genome.

The new SARS-CoV-2 is a single-stranded RNA-containing virus, belonging to the Coronaviridae family to the Beta-CoV B lineage. The virus belongs to pathogenicity group II, as well as some other representatives of this family (SARS-CoV virus, MERS-CoV), and is presumably a recombinant virus between bat coronavirus and coronavirus of unknown in origin. The emergence of COVID-19 has given healthcare professionals the task of providing rapid diagnosis and patient care. Currently, the intensive study of clinical and epidemiological disease features continues, as well as the development of new prevention and treatment means. The most common clinical syndrome of the new coronavirus infection variant is bilateral pneumonia (viral diffuse alveolar damage with microangiopathy), and acute respiratory distress syndrome (ARDS) development has been registered in 3-4% of patients. In some patients hypercoagulation syndrome with thrombosis and thromboembolism develops, other organs and systems (central nervous system, myocardium, kidneys, liver, gastrointestinal tract, endocrine and immune systems) are also affected, sepsis and septic shock may develop.¹ Early diagnosis of severe acute respiratory infection (atypical pneumonia) caused by SARS - CoV-2 is extremely important for correct and timely diagnosis and treatment.

¹ Temporary guidelines "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19). Version 16 (18.08.2022)" (approved by the Ministry of Health of the Russian Federation)

The scope of the reagent kit: clinical laboratory testing of infectious diseases.

Indications for use: SARS-CoV-2 RNA detection by RT-PCR is recommended for patients with clinical symptoms of a respiratory disease suspected of infection caused by SARS-CoV-2, especially those arriving from epidemiologically unfavorable regions immediately after initial examination, as well as for contact persons ¹.

Contraindications for use: when used by specially trained personnel and taking into account the intended use, there are no contraindications, except in cases when biomaterial collection cannot be carried out for medical reasons.

Population, demographic aspects of the kit use: the detection of SARS-CoV-2 RNA is carried out in patients with clinical symptoms of respiratory disease, suspected of infection with SARS-CoV-2 virus, especially those arriving from epidemiologically unfavorable regions immediately after the initial examination, as well as contact persons ².

Sterility: the product is not sterile.

1. Intended use

Intended use: Cito-CoV-2-Test reagent kit is designed for qualitative detection of SARS-CoV-2 (2019-nCoV, COVID-19) strain genomic RNA specific region, a fragment of *N* gene encoding the nucleocapsid phosphoprotein, by single-stage reverse transcription – multiplex allele-specific polymerase chain reaction with hybridization-fluorescence detection in clinical samples (nasopharyngeal, oropharyngeal swabs) in order to diagnose severe acute respiratory viral infection caused by SARS-CoV-2 in patients with clinical symptoms of respiratory disease, as well as contact persons. A separate RNA isolation kit is not required, since the kit includes RT-PCR buffer that provides viral particles lysis and it is resistant to inhibitors.

Functional purpose: the results obtained can be used to help diagnose severe acute respiratory viral infection caused by SARS-CoV-2. The results are taken into account in the comprehensive disease diagnosis.

Potential consumers of a kit

For research use only.

2. Method principle

Method

Single-stage reverse transcription - multiplex allele-specific polymerase chain reaction (RT-PCR) in real time with hybridization-fluorescence detection, which does not require RNA isolation procedure (direct RT-PCR-RT)

Test sample type

The assay material is a patient's clinical material taken from the upper respiratory tract (a nasopharyngeal, oropharyngeal swab).

Detection principle

SARS-CoV-2 nucleic acids detection is based on the use of a reverse transcription reaction method followed by a real-time polymerase chain reaction in a single test tube.

RT-PCR buffer contains all the basic reagents, including dNTP, genetically modified MMLV revertase with suppressed RNase H activity and increased thermal stability, which allows to increase cDNA output, antibody-inactivated DNA polymerase, which does not have enzymatic activity under reaction mixture preparation and reversion conditions, which leads to increased specificity and sensitivity, RT-PCR buffer content allows perform RT-PCR in the presence of a large inhibitors number, as well as provides viral particles lysis.

The primer mix contains fluorescently labeled oligonucleotide probes that hybridize with a complementary region of the amplified DNA target and are destroyed by *Taq* polymerase, as a result the fluorescent dye and quencher are separated, and the fluorescence intensity increases. This allows the specific amplification product accumulation to be recorded by measuring the fluorescent signal intensity in real time.

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

The channel corresponding to the fluorophore	
FAM	HEX
SARS-CoV-2	ICS

ICS allows evaluate the RT-PCR correctness and reaction inhibition degree.

Method limitations

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

Damage to the packaging integrity during transportation.

Use of an expired kit or kit storage conditions violation.

Storage and transportation conditions violation.

RT-PCR reaction time ranges from 80 to 100 minutes, depending on the cycler model used (excluding sample preparation).

3. Reagent kit components

Configuration forms

Cito-CoV-2-Test reagent kit is available in one configuration form.

Number of test samples

Each Cito-CoV-2-Test reagent kit is designed to perform 96 RT-PCR reactions, it equates to detection of 94 test samples, negative and positive control samples, or 32 single test samples detections with negative and positive control samples in each test.

Kit components

Table 1 – Cito-CoV-2-Test reagent kit components:

No.	Reagent name	Description	Quantity, volume
1	RT-PCR buffer	Transparent colorless Liquid	1 tube, 480 µl
2	Primer mix	Transparent colorless liquid, may have a shade of pink	1 tube, 480 µl
3	PC	Transparent colorless liquid	1 tube, 480 µl
4	NC	Transparent colorless liquid	1 tube, 480 µl
5	ICS	Transparent colorless liquid	1 tube, 940 µl

Note: Operational documentation (instructions for use and quality certificate) is not included in the product, but is included in the product delivery set. A reagent kit, to ensure compliance with transportation conditions is placed in a reusable

polyurethane foam thermal container with prepared ice packs for temporary storage and transportation. The thermal container, the instructions for use and the quality certificate for each batch of products supplied are placed into an individual packaging.

RT-PCR buffer is ready for use and contains a warm start revertase, a thermally stable hot start DNA polymerase, dNTP and an optimized buffer that allows RT-PCR to be performed in the presence of a large number of inhibitors.

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

1. Primers and a probe specific to a fragment of the *N* gene encoding the nucleocapsid phosphoprotein – the SARS-CoV-2 strain genomic RNA specific part. The *N* gene fragment, used as a target in this test system, is recommended by FDA for the coronavirus detection.¹ Detection is carried out in the FAM channel.
2. Primers and a probe to the internal control sample. Detection is in the HEX channel.

Reaction in HEX channel makes it possible to evaluate the RT-PCR correctness in the inhibitors presence in the sample. If there is no reaction, the result should be considered unreliable, and in this case, it is recommended to retake the material for assay for this test sample.

Positive control sample (PC) is ready for use and it is a plasmid vector with DNA synthetic insertions, complementary RNAs of the internal control sample and SARS-CoV-2 *N* gene fragment.

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

Internal control sample (ICS) is ready for use and it is an NA sample.

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

4. Reagent kit characteristics

4.1 Technical and functional characteristics

Table 2. Cito-CoV-2-Test reagent kit

Indicator name	Characteristics and standards
1. Technical characteristics	
1) Appearance	
RT-PCR buffer	Transparent colorless liquid
Primer mix	Transparent colorless liquid, may have a shade of pink
PC	Transparent colorless liquid
NC	Transparent colorless liquid
ICS	Transparent colorless liquid
1.2. Completeness	According to clause 1.4 TS 21.20.23-028-97638376-2020
1.3. Labelling	According to clause 4 TS 21.20.23-028-97638376-2020
1.4. Packaging	According to clause 5 TS 21.20.23-028-97638376-2020
2. Functional characteristics	
Positive result with PC	Fluorescence signal growth registration in in tubes with PC in the FAM channel $Ct \leq 30$ and in the HEX channel $Ct \leq 30$.
Negative result with NC	In tubes with NC in the FAM and HEX channels, Ct is not indicated or >40
Reaction course with ICS + NC	Fluorescence signal growth registration in the HEX Ct channel is ≤ 30 and there is no fluorescence accumulation diagram in the FAM channel.
Reaction course with SenC	In tubes with SenC in the FAM and HEX channels in all repetitions (at least 6) $Ct \leq 40$ and with a standard deviation value in SenC repeats is no more than 3%
Reaction course with SC	There is no fluorescence accumulation diagram in FAM and HEX channels.
Reaction course with SC + ICS	Fluorescence signal growth registration in the HEX Ct channel is ≤ 30 and there is no fluorescence accumulation diagram in the FAM channel.
Limit of detection	Reaction with SARS-CoV-2 at a concentration of 500 copies per 1 ml of biomaterial: fluorescence signal growth registration in the FAM channel $Ct \leq 35$ and in the HEX channel is <30 .

Note:

1) the Panel of inactivated respiratory pathogens for analytical specificity testing of in vitro diagnostic medical devices, SARS-CoV-2 coronavirus, strain GK2020/1, cat. No. AA006, manufactured by Federal State Budgetary Institution GAMALEYA NATIONAL CENTER of epidemiology and microbiology of the Ministry of Health of Russia is used to control the limit of detection.

2) during the control PCR, as SenC and SC are used:

- a plasmid mixture with synthetic insertions of sequences, complementary to CoV-2 targets and an internal control sample in a TE buffer (10 mM Tris, 1 mM EDTA) at a concentration of 500 copies in 1 ml of each is used as a control sample for determining sensitivity (SenC);

- a solution of human genomic DNA isolated from the jurkat cell line at 3,335 copies per 5 µl (667 copies/µl) concentration is used as a specificity control sample (SC).

4.2 Characteristics of analytical efficiency

4.2.1 Analytical specificity

It is specific to N gene fragment, encoding the nucleocapsid phosphoprotein – SARS-CoV-2 strain genomic RNA specific part.

4.2.1.1 Confirmation of specificity in silico

The analytical specificity of N gene target fragment encoding SARS-CoV-2 nucleocapsid phosphoprotein was confirmed *in silico* using the BLAST resource (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

4.2.1.2 In vitro specificity confirmation using certified control materials

To evaluate the possibility of SARS-CoV-2 virus RNA detection, a standard NIBSC sample was used:

- The first WHO International Standard for SARS-CoV-2 RNA testing, NIBSC code: 20/146.

WHO International Standard after reactivation was tested in two rounds in each testing cycle.

Using PCR reactions series, the International Standard study was carried out with Cito-CoV- 2-Test reagent kit.

Rotor-Gene Q (QIAGEN) cyclor, Registration certificate No. FSZ 2010/07595 dated 10.08.2010, was used for the assay.

Based on the assay results of the WHO International Standard the first WHO International Standard for SARS-CoV-2 RNA testing, NIBSC code: 20/146, Cito-CoV-2-Test reagent kit specificity in relation to SARS-CoV-2 (2019-nCoV, COVID-19) strain RNA has been confirmed.

4.2.1.3 Analytical specificity: cross-reactivity evaluation

Analytical specificity studies: cross-reactivity evaluations were carried out in the RNA/DNA assay of the following microorganisms/strains:

– strains from ATCC collection (American Type Culture Collection, USA):

Streptococcus pneumoniae (ATCC® 49619™),

Pseudomonas aeruginosa (ATCC® 9027™),

Mycoplasma pneumoniae, Strain PI 1428 (ATCC® 29085™),

Chlamydomphila pneumoniae, Strain CM-1 (ATCC® VR-1360™),

Legionella pneumophila subsp. pneumophila, Strain Philadelphia 1 (ATCC® 33152™),

Staphylococcus epidermidis, FDA Strain PCI 1200 (ATCC® 12228™),

Human Respiratory Syncytial Virus, Strain 9320 (ATCC® VR-955™),

Human Respiratory Syncytial Virus, Strain A-2 (ATCC® VR-1540™),

Human Parainfluenza Virus 1, Strain C35 (ATCC® VR-94™),

Human Parainfluenza Virus 2, Strain Greer (ATCC® VR-92™),

Human Parainfluenza Virus 3, Strain C243 (ATCC® VR-93™),

Human Rhinovirus 17, Strain 33342 (ATCC® VR-1663™),

Human Adenovirus 1, Strain Adenoid 71 (ATCC® VR-1™),

Human Coronavirus, Strain OC43 (ATCC® VR-1558™),

Human Coronavirus, Strain 229E (ATCC® VR-740™),

at a concentration of not more than 1×10^9 GE/ml and not less than 1×10^6 GE/ml;

– strains from State Collection of Pathogenic Microorganisms:

Haemophilus influenzae 423,

Streptococcus pyogenes Dick – I,

Bordetella pertussis 703 L 6,

Mycobacterium Bovis Ravenel №700204

at a concentration of not more than 1×10^9 GE/ml and not less than 1×10^6 GE/ml;

– strains from the collection of Research Institute of Influenza named after A.A.Smorodintsev:

A/St. Petersburg/NIIG-252/19 (Influenza A virus (H3N2)),

A/Kaliningrad/75/19 (Influenza A virus (H1N1)pdm09),

B/Washington/02/19 (Influenza B virus lineage Victoria),
B/Yakutsk/NIIG-06/2019 (Influenza B virus lineage Yamagata) at a concentration of not more than 1×10^9 GE/ml and not less than 1×10^7 GE/ml.

The purified DNA/RNA of the above-mentioned microorganisms was tested using Cito-CoV-2-Test reagent kit. According to the test results, no nonspecific reactions were detected.

4.2.2 Analytical sensitivity: limit of detection (LOD)

In accordance with GOST R 51352-2013 and taking into account CLSI EP-17A2 international recommendations, the detection limit (LOD) was determined by testing WHO International Standards dilutions:

- The first WHO International Standard for SARS-CoV-2 RNA testing, NIBSC code: 20/146.

in the range of the estimated detection limit in 1 ml of test biological sample: 100 copies/ml, 200 copies/ml, 300 copies/ml, 400 copies/ml, 450 copies/ml, 500 copies/ml, 550 copies/ml. Each of the 7 dilutions was tested with Cito-CoV-2-Test kit for 3 different days in 30 repetitions to calculate the positive results percentage. The results were determined in accordance with CLSI EP-17A2 by international recommendations by probit analysis.

To conduct a PCR assay with Cito-CoV-2-Test reagent kit, cyclers recommended by the test reagent kit manufacturer were used:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany);
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA).

In accordance with **CLSI EP-17A2 recommendations, statistical processing of the obtained results was carried out** using probit analysis. To calculate SARS-CoV-2 RNA limit concentration with 95% confidence probability, a probit value (Y axis) 6.64 was used (1.64 for the 95% limit, +5 for the probit scale).

According to the assay results, SARS-CoV-2 RNA detection limit in 1 ml biosamples with a detection rate 95% was 494 copies/ml (95% CI: 492.57-495.43) for DTprime cycler, 490 copies/ml (95% CI: 488.57-491.43) for CFX 96, and for Rotor-Gene Q – 496 copies/ml (95%CI: 494.57-497.43), Quant Studio 5 – 494 copies/ml (95%CI: 492.57–495.43).

4.2.3 Precision under repeatability conditions

To evaluate precision under repeatability conditions, a positive control sample, an internal control sample of 10 repetitions of each were used.

Repeatability data is obtained inside the laboratory for specific equipment and within a specific reagent kit batch.

To evaluate precision under repeatability conditions, the arithmetic mean of the sample, variance, standard deviation and coefficient of variation are calculated based on the values obtained in control samples repetitions.

The assay results showed that the coefficient of variation under the kit repeatability conditions is not more than 3%.

4.2.4 Precision under reproducibility conditions

The test system reproducibility is evaluated similarly to the calculation of precision under repeatability conditions (Section 4.2.3.), however, different batches of the reagent kit are used for testing, reactions are performed in different laboratories, by different operators, on different days, on different PCR cyclers (Reproducibility Unit 1, Reproducibility Unit 2, Reproducibility Unit 3, Reproducibility Unit 4).

When performing precision assay under reproducibility conditions, complete intra-assay, inter-assay and inter-series reproducibility was observed, the coefficient of variation does not exceed 3%.

4.2.5 Control samples metrological traceability – PC, SenC

By parallel testing a positive control sample PC and a control sample for sensitivity detection (SenC), their metrological traceability to “The first WHO International Standard for SARS-CoV-2 RNA Assays”, NIBSC code: 20/146, was confirmed.

Based on the calibration and standardization process obtained results, it can be concluded that SenC assigned concentration is ~ 500 copies/ml, PC ~ 200,000 copies/ml (200 copies/ml). The uncertainty for PC is - 0.02 log₁₀ copies/ml and for SenC is 0.02 log₁₀ copies/ml.

4.3 Clinical efficiency characteristics

4.3.1 The kit diagnostic characteristics assay

125 clinical samples of upper respiratory tract swabs from patients with suspected SARS-CoV-2 infection were used for clinical trials.

For these clinical material samples SARS-CoV-2 RNA presence was detected during the treatment and diagnostic process using the registered ‘Reagent kit for qualitative detection of coronavirus (SARS-CoV-2) RNA

by real-time RT-PCR method “CoV-2-Test” manufactured by TestGene LLC, Russia, batch 202109-316, shelf life 2022-09 (№ RZN 2020/10364 dated 15.05.2020).

In 65 patient samples provided SARS-CoV-2 RNA presence was detected by CoV-2-Test reagent kit and in 60 samples - was not detected.

Each sample was tested in two series using Cito-CoV-2-Test reagent kit manufactured by TestGene LLC and CoV-2-Test comparison kit manufactured by TestGene LLC, Russia.

For PCR assay cyclers, recommended by the test reagent kit manufacturer, were used:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany);
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA).

When testing 125 samples - swabs from the upper respiratory tract with the tested reagent kit in two series (250 observations) for all four cyclers, it was determined that 64 samples were true positive (in two series - 128 observations), 1 sample was false negative (in two series - 2 observations) and 60 samples were true negative (in two series - 120 observations) for SARS-CoV-2 RNA.

Confidence intervals (CI) of diagnostic characteristics are calculated using the Clopper and Pearson Confidence Interval (Clopper-Pearson Confidence Interval; Clopper, C., & Pearson, E. (1934). *The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial*. *Biometrika*, 26(4), 404-413. doi:10.2307/2331986). The kit diagnostic characteristics were calculated with 95% confidence probability.

The results of the kit diagnostic characteristics assay are shown in the table.

Table 3.

Test material type	Cycler	Number of samples	Number of observations	Diagnostic sensitivity with 95% confidence probability %	Diagnostic specificity with 95% confidence probability %
Swab from the upper respiratory tract (nasopharynx and oropharynx)	DTprime	125	250	98,4% (95% CI: 94,5%-99,8%)	100% (95% CI: 96,9%-100%)
	CFX 96	125	250	98,4% (95% CI: 94,5%-99,8%)	100% (95% CI: 96,9%-100%)
	Rotor-Gene Q	125	250	98,4% (95% CI: 94,5%-99,8%)	100% (95% CI: 96,9%-100%)
	QuantStudio 5	125	250	98,4% (95% CI: 94,5%-99,8%)	100% (95% CI: 96,9%-100%)

4.3.2 Evaluation of interfering substances effects

The effect of potentially interfering substances on Cito-CoV-2-Test reagent kit performance has been tested for potentially interfering substances that may originate from the following external and internal sources:

- 1) substances used in a patient' treatment (for example, medicines);
- 2) substances found in specific sample types - in this case, clinical sample contamination with a biological agent (mucin, hemoglobin) can inhibit PCR.

Table 4. Conducted assay results analysis.

Interfering substance (Type of substances)	Maximum concentration	Result	
		False positive results	False negative results
Hemoglobin	10%	0/3	0/3
Mucin	5%	0/3	0/3
Ibuprofen	0.04 mg per ml	0/3	0/3
Ambroxol hydrochloride	0.003 mg per ml	0/3	0/3
Bromhexine hydrochloride	0.016 mg per ml	0/3	0/3

Lopinavir	0.016 mg per ml	0/3	0/3
Ritonavir	0.004 mg per ml	0/3	0/3
Interferon alpha	0.2 units per ml	0/3	0/3
Paracetamol	0.065 mg per ml	0/3	0/3
Pheniramine maleate	0.04 mg per ml	0/3	0/3
Phenylephrine hydrochloride	0.002 mg per ml	0/3	0/3

Based on the clinical assays results, potentially interfering substances encountered during the biological material sampling procedure, evaluated at concentrations expected to be encountered during normal Cito-CoV-2-Test reagent kit use, do not affect the assay result.

4.3.3 Kit reproducibility and repeatability evaluation

4.3.3.1 Repeatability evaluation (within a single testing) was performed on three positive clinical samples and one negative sample, in five repetitions.

Kits from one packaging and one batch were used to evaluate the kit repeatability. The assay was carried out by the same operator at the same time.

The calculated coefficient of variation does not exceed 3%.

4.3.3.2 Kit reproducibility (between series) and repeatability (within a single testing) were evaluated on 3 positive clinical samples and one negative sample, in five repetitions.

The calculated coefficient of variation does not exceed 3%.

4.3.4 Cross-reactivity assay

Cross-reactivity of "Reagent kit for qualitative detection of coronavirus (SARS-CoV-2) RNA by direct RT-PCR-RT "Cito-CoV-2-Test", manufactured by TestGen LLC, Russia, was evaluated according to the manufacturer's instructions by testing 25 samples of oropharyngeal swabs containing no SARS-CoV-2 RNA from patients with confirmed respiratory pathogens: respiratory syncytial virus (RSV) - 11 samples, parainfluenza viruses - 4 samples, rhinoviruses - 3 samples, adenoviruses - 5 samples, human metapneumoviruses - 2 samples.

Each sample was tested in three rounds.

The results showed that no cross-reactivity was observed when 25 oropharyngeal swab samples containing no SARS-CoV-2 RNA were tested from patients with confirmed respiratory pathogens: respiratory syncytial virus (RSV) – 11 samples, parainfluenza viruses – 4 samples, rhinoviruses – 3 samples, adenoviruses – 5 samples, human metapneumoviruses - 2 samples, no cross-reactivity was observed.

5. Risks associated with the reagent kit use

The border risk zone includes the following hazards:

1. Loss of functional properties of the reagents included in the kit due to transportation, storage or usage under inappropriate conditions,
2. Contamination of reaction mixtures with test samples with contents from a test tube or PCR products,
3. Failure to comply with the requirements for sample preparation, testing and disposal (failure to comply with the requirements for clinical material preliminary inactivation);
4. The use of an unsuitable kit (use after the expiry or in case of damaged packaging).

The cumulative residual risk of using “Reagent kit for qualitative detection of coronavirus RNA (SARS-CoV-2) by direct RT-PCR-RT "Cito-CoV-2-Test" is acceptable, the benefit of its use exceeds the risk.

6. Safety precautions

The class, depending on the potential risk of use, is 3 in accordance with the medical devices nomenclature classification approved by the order of the Ministry of Health of the Russian Federation dated 06.06.2012 N 4h.

ATTENTION! The clinical material must be pre-inactivated by heating at 70°C for 5 minutes in the working area ¹².

All components and reagents included in Cito-CoV-2-Test reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "Occupational safety standards system. Harmful substances. Classification and general safety requirements".

¹² "Temporary guidelines "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19). Version 15 (22.02.2022) (approved by the Ministry of Health of the Russian Federation)

The reagents included in Cito-CoV-2-Test kit have low vapor

pressure and exclude the possibility of inhalation poisoning.

The reagents included in Cito-CoV-2-Test kit are non-toxic, since they are prepared by mixing individual non-toxic components.

Specialists who have given written consent and have been instructed by employees of Rospotrebnadzor laboratories who have a sanitary and epidemiological conclusion to work with pathogens of human infectious diseases of pathogenicity group II are allowed to work with test systems for COVID-19 diagnostics in the laboratory of organizations.

Clinical material sampling and its packaging is carried out by an employee of a medical organization trained in the requirements and rules of biological safety when working and collecting material suspected of infection with microorganisms of pathogenicity group II. Each sample of the material is placed in a separate transport container, ensuring the requirements in accordance with the table of these guidelines.

All samples collected for laboratory testing should be considered as potentially infectious, and medical personnel who collect or transport clinical samples must strictly comply with the requirements of biological safety as when working with microorganisms of pathogenicity group II.

Samples transportation must be carried out in accordance with the requirements of sanitary legislation in relation to microorganisms of the pathogenicity group II.

All samples obtained for laboratory testing should be considered potentially infected, and when working with them, the requirements of SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases" should be taken into account. Healthcare workers who collect or transport clinical samples to the laboratory should be trained in safe biomaterial handling practices, strictly observe precautions and use personal protective equipment (PPE).

Work with material infected or suspected of being infected with SARS-CoV-2 is carried out in accordance with the requirements of sanitary and epidemiological rules for the safety of working with microorganisms of pathogenicity groups I-II (SanPiN 3.3686-21), temporary methodological recommendations "Prevention, diagnosis and treatment of new coronavirus infection (2019-nCoV), as well as an Information Letter from the Federal Service for Surveillance of Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) dated 21.01.2020 No. 02/706-2020-27 "On the direction of temporary recommendations on the organization of laboratory diagnostics of a new

coronavirus infection (2019-nCoV)."

It is necessary to simultaneously ensure and comply with the biological safety rules and requirements for the organization and conduct of these works by personnel in order to prevent premises and equipment contamination with nucleic acids and (or) amplicons of the tested samples.

The work should be carried out in a laboratory performing molecular biological (PCR) assays of clinical material in compliance with sanitary and epidemiological rules SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, 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." Follow methodological recommendations MU 287-113.

The following requirements should always be met when working:

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

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is unacceptable to open the tubes and splash the contents, as this can lead to contamination of the laboratory area, equipment and reagents with PCR products.

1. Use the kit strictly for its intended purpose, according to these instructions;
2. Do not use the kit after the expiration date;
3. Avoid contact with skin, eyes and mucous membranes. In case of contact, rinse immediately the affected area with water and seek medical assistance.

The necessary precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload, sources of thermal inflammation are not provided.

The kit contains no substances of human or animal origin with a potential infectious nature, therefore, precautions against any special,

unusual risks during product use or sale are not provided.

7. Required equipment and materials

Work with the reagent kit for RT-PCR Cito-CoV-2-Test is carried out in working area 2 (for NA isolation) (MU 1.3.2569- 09).

Equipment for carrying out RT-PCR reaction:

1. Class II and III biological safety PCR-cabinet (for example, BMB-II – Laminar-S-1,2, Lamsystems, Russia).
2. Vortex (for example, TETA-2, Biocom, Russia).
3. A set of electronic or automatic variable volume dispensers (for example, Eppendorf, Germany).
4. Refrigerator from 2°C to 8°C with freezer less than -16°C.
5. Cycler ³ with real-time fluorescence detection in channels corresponding to FAM and HEX fluorophores: CFX96 (BioRad, USA), DTprime, (DNA-Technology, Russia) Rotor-Gene Q (Qiagen, Germany), QuantStudio 5 (Thermo Fisher Scientific, USA).

Materials and reagents not included in the kit:

ATTENTION! When working, it is necessary to use only disposable sterile plastic consumables that with "RNase-free" label.

1. Disposable pipette tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (for example, Axygen, USA).
2. A separate gown and disposable gloves without talcum powder.
3. Container with disinfectant solution.
4. Test tube racks for 0.1 or 0.2 ml tubes or for 0.1 or 0.2 ml stripped tubes (for example, InterLabService, Russia).
5. To take a swab from the nasopharynx and oropharynx, it is recommended to use a "Medical disposable sterile probe according to TS 32.50.13-002-28731857-2020", produced by PharMedoPolis RT LLC, Russia (registration certificate No. RZN 2021/13989 dated 12.04.2021).

³ Cyclers must be maintained, calibrated and used according to the manufacturer's recommendations. Use of this kit in an uncalibrated device may effect the test performance.

8. Test samples

Test sample type

The biological material for the assay is a swab from the nasopharynx and pharynx (oropharynx).

ATTENTION! As tubes with transport medium, use ONLY sterile 1.5-2.0 ml Eppendorf type tubes containing 500 µl of sterile saline solution (or phosphate buffered saline (PBS)). The use of transport medium with different components may result in an invalid result due to the reaction inhibition.

ATTENTION! The use of 5-15 ml centrifuge tubes use can lead to false positive results due to contamination when transferring clinical material to a test tube for RT-PCR. If it is impossible to refuse 5-15 ml centrifuge tubes, it is recommended to pre-transfer the biomaterial to an Eppendorf-type 1.5-2.0 ml test tube using long tips per 1000 µl, then add the biomaterial to RT-PCR with 10-20 µl tip from an Eppendorf-type 1.5-2.0 ml test tube.

ATTENTION! Avoid repeated samples freezing and thawing.

8.1 Biological material sampling procedure

ATTENTION! Diagnostic material sampling, its packaging, labelling and transportation are carried out in accordance with the requirements and rules for working with materials potentially infected with pathogens of pathogenicity group II, their storage and transportation according to MU 1.3.2569-09 "Organization of laboratories work using NAAT when working with material containing microorganisms of pathogenicity groups I - IV" and Methodological recommendations of Rospotrebnadzor MU 3.1.0169-20 "Laboratory diagnostics of COVID-19".

A medical worker, performing the diagnostic material collection, its labelling and packaging, must be instructed on sanitary and epidemiological requirements and biological safety rules when working with patients, potentially infected with microorganisms of pathogenicity group II.

Material sampling

Nasopharyngeal swabs

Nasal swab is taken with dry sterile cotton swabs on a plastic base. Insert a swab with a light movement along the outer nasal wall to 2-3 cm depth to the lower concha. Then slightly lower the swab, insert into the

lower nasal passage, make a rotational movement and remove the swab along the outer nasal wall.

Oropharyngeal swabs

Swabs are taken with dry cotton swabs on a plastic base with rotational movements from the surface of the tonsils, palatine arches and the posterior wall of the oropharynx.

After taking the material, place the swab (the applied part of the probe with a cotton swab) in a sterile disposable Eppendorf-type tube with 500 µl of sterile saline solution (or phosphate buffered saline (PBS)) and carefully brake off the plastic rod leaving up to 0.5 cm from the applied part, leaving the applied part of the probe with the material inside. Close the tube tightly with a lid.

ATTENTION! To increase the virus concentration, nasopharyngeal and oropharyngeal swabs should be placed in the same tube.

Transportation, storage and disposal conditions of initial clinical material:

The temperature during transportation should be +2° - +8°C. The sample storage time before the test should not exceed 5 days at +2° - +8°C, it may be longer at -20°C or -70°C.

Hermetically sealed containers with samples are transported to the laboratory in special containers/dressing drums. Referrals and other documentation in hard copy are transferred in a separate plastic bag.

If it is necessary to send samples to the laboratory of another medical institution, the requirements for the transfer of infectious materials of pathogenicity group II (SP 1.2.036-95 "Order of calculation, storage, transfer and transportation of the microorganisms of pathogenicity groups I - IV") are fulfilled.

Test tubes / containers with samples together with the lid are sealed with various sealants (paraffin, parafilm, etc.); the container is labelled. Place the samples of each patient in an individual sealed bag with an absorbent material and pack additionally in a common sealed bag.

Two or more samples from the same patient can be packed in one plastic bag. It is forbidden to pack clinical material samples from different people in the same packaging.

Place the packaging with containers in a hermetically sealed container for the biological material transportation. Place the container in a foam thermocontainer with ice packs. Sealed and label the transport container. It is recommended to place a disposable indicator in the container that monitors temperature compliance from +2° to +8°C.

Place the accompanying documents in an individual packaging separately from the biological material and fasten securely to the outside of the container.⁴

8.2 Interfering substances and restrictions on the test material use

The effect of potentially interfering substances on Cito-CoV-2-Test reagent kit performance has been tested for potentially interfering substances that may originate from the following external and internal sources:

- 1) substances used in a patient's treatment (for example, medicines);
- 2) substances found in specific sample types - in this case, clinical sample contamination with a biological agent (mucin, hemoglobin) can inhibit PCR.

The tested concentrations of interfering substances, which are expected to occur during Cito-CoV-2-Test reagent kit normal use, are shown in Table 5.

⁴ "Temporary guidelines "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19). Version 15 (22.02.2022) (approved by the Ministry of Health of the Russian Federation)

Table 5.

Type	Material	The active component	Concentration
Endogenous	Biological agents	Mucin	2% m/v
		Hemoglobin	0.01% v/v
Exogenous	Over-the-counter nasal drops	Phenylephrine	15% v/v
	Over-the-counter nasal gel	Sodium chloride	5% v/v
	Over-the-counter nasal spray 1	Cromolyn	15% v/v
	Over-the-counter nasal spray 2	Oxymetazoline	15% v/v
	Over-the-counter nasal spray 3	Fluconazole	5% m/v
	Throat lozenges	Benzocaine, menthol	0.15% m/v
	Over-the-counter homeopathic nasal spray 1	Galphimia glauca, Sabadilla	20% v/v
	Over-the-counter homeopathic nasal spray 2	Zinc gluconate	5% m/v
	Over-the-counter homeopathic nasal spray 3	Alkalol	10% v/v
	Over-the-counter homeopathic nasal spray 4	Fluticasone propionate	5% v/v
	Sore throat spray with phenol	Phenol	15% v/v
	Antiviral Medicine	Tamiflu (oseltamivir phosphate)	0.5% v/v
	Antibiotic, nasal ointment	Mupirocin	0.25% v/v
	Antibacterial, systemic	Tobramycin	0.0004% m/v

Based on the assay results, these substances do not have an interfering effect on the kit operation and do not lead to PCR inhibition at concentrations not exceeding the permissible ones.

To reduce the PCR inhibitors number, it is necessary to follow the rules for clinical material sampling.

Limitations on the test material use:

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

- it is not allowed to use samples contaminated with extraneous biological material.

9. Testing procedure

Installation, assembling, adjustment, calibration of the kit for commissioning is not required.

ATTENTION! When working with RNA, it is necessary to use only disposable sterile plastic consumables with "RNase-free" label. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

ATTENTION! The clinical material must be pre-inactivated by warming up at 70°C for 5 minutes in the working area ⁵.

ATTENTION! The reaction mixture components should be mixed according to Table 4 directly in PCR tubes before the test.

9.1. Carrying out RT-PCR

Kit components preparation for RT-PCR

1. Thoroughly mix the contents of the test tubes with the prepared clinical material, NC, RT-PCR buffer, ICS, Primer mix and PC, turning each tube 10 times or by stirring on a vortex at low speed for 3-5 seconds, and then discharge the drops from the tube lids by short centrifugation.

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

⁵ Temporary guidelines "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19). Version 15 (22.02.2022) (approved by the Ministry of Health of the Russian Federation)

Before preparing the reactions, it is necessary to wet clean the PCR box, as well as the equipment and materials contained in it with disinfectants suitable for use in PCR laboratories, turn on the UV lamp for 20-30 minutes.

Total reaction volume is 25 µl.

ATTENTION! It is forbidden to change the reaction volume. When the volume changes, the method sensitivity decreases dramatically!

To carry out one reaction, you need:

1. RT-PCR buffer – 5 µl,
2. Primer mix – 5 µl,
3. Sample (PC, NC, clinical material) – 5 µl,
4. Internal control sample - 10 µl.

RT-PCR protocol

The reaction tubes must be prepared according to Table 5 in the following order:

1. Label 0.1-0.2 ml tubes for RT-PCR, one for each test sample, one tube for PC and one tube for NC (Table 6).

Table 6 – An example of the test tubes layout and PCR components adding

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
Sample, µl	5	5	5	5	-	-
ICS, µl	10	10	10	10		
PC, µl	-	-	-	-	15	-
NC, µl	-	-	-	-		15

2. Add 5 µl of RT-PCR buffer into each tube.

3. Add 5 ml of Primer mix into each tube.

4. Add 5 µl of the sample into appropriate test tubes for test samples.

Do not add the sample into the tubes for PC and NC.

5. Add 10 µl of ICS into appropriate test tubes. Do not add internal control sample into the tubes for PC and NC.

6. Add 15 µl of PC into the appropriate test tube.

7. Add 15 µl of NC into the appropriate test tube.

8. To discharge drops from the walls, centrifugate the tubes for 1-3 seconds on a vortex microcentrifuge.

9. Place the test tubes in the reaction module of the real-time PCR

device. It is recommended to place the test tubes in the center of the thermoblock to evenly press the test tubes with a heating lid.

10. Program the device to perform the corresponding program for fluorescent signal amplification and detection, following the instructions for the device used. RT-PCR protocol is shown in Table 7.

Table 7 – RT-PCR protocol for Cito-CoV-2-Test reagent kit

Stage	Temperature, °C	Time, min:sec	Detection channels	Total number of cycles
1	52	25:00		1
2	95	02:00		1
3	95	00:15		5
	64	00:20		
4	95	00:15		45
	64	00:20	FAM, HEX	

11. Specify the samples number and identifiers, mark the tubes location on the thermoblock matrix in accordance with their layout.

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

13. Start RT-PCR with of a fluorescent signal detection.

14. At the end of the program, start analyzing the results.

10. Result registration and interpretation

Results registration is carried out automatically upon RT-PCR completion with the used device software. The result interpretation is performed according to Ct values of ICS (HEX channel) and Ct of the coronavirus target (FAM channel).

First, the reaction passage and Ct values in the control samples are evaluated. The result interpretation in the test samples begins only with the correct PC and NC passage.

Result interpretation in control samples

The following results should be obtained for negative and positive control samples (Table 8).

Table 8 – Assay results for negative and positive control samples

The added material	Detection channel	
	FAM (coronavirus)	HEX (ICS)
NC	Ct >40 or absent	Ct >40 or absent
PC	Ct ≤30	Ct ≤30

When obtaining values for a negative control sample that differ from those indicated in Table 8, the results of the entire tested series are considered unreliable. In this case, special measures should be taken to eliminate possible contamination.

When obtaining values for a positive control sample that differ from those indicated in Table 8, repeated amplification of the entire samples batch is required.

Result interpretation in test samples

Result interpretation principle is shown in Table 9 and is performed in the following order:

1. The test quality is determined by the Ct value for the HEX channel, which should not exceed 30.
2. The reaction result is evaluated using the FAM channel.

Table 9 – Result interpretation principle.

The value of the threshold cycle (Ct) by channels		Result
FAM	HEX	
Absent	Ct ≤30	SARS-CoV-2 RNA not detected
Ct ≤35	not considered ⁶	SARS-CoV-2 RNA detected
Ct >35 or absent	Ct >30 or absent	The result is invalid
Ct >35	Ct ≤30	The result is doubtful

⁶ At high SARS-CoV-2 RNA concentrations, HEX channel output may occur in late cycles or be absent.

Result interpretation principle:

SARS-CoV-2 RNA is not detected "-" if Ct in the HEX channel is not more than 30, and Ct in the FAM channel is not indicated (there is no fluorescence accumulation diagram).

SARS-CoV-2 coronavirus RNA is detected "+" if the Ct in the FAM channel is not more than 35. The result of the HEX channel is not taken into account.

The test result is invalid "?" if Ct in the FAM channel is not indicated (there is no fluorescence accumulation diagram) or more than 35, and Ct in the HEX channel is not indicated (there is no fluorescence accumulation diagram) or more than 30.

The reason for obtaining an invalid result may be a low RNA concentration; a high inhibitor concentration in a clinical sample; incorrect implementation of the test protocol; non-compliance with the RT-PCR temperature regime, etc.

The test result is doubtful if Ct in the FAM channel is more than 35, and Ct in the HEX channel is not more than 30.

In case of an invalid and doubtful result, a conclusion is not issued, it is necessary to retake the biomaterial from the patient and retest it.

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

11. Kit storage, transportation and usage conditions

Storage

Cito-CoV-2-Test reagent kit in the manufacturer's packaging should be stored at a temperature from -16 to -24°C during the entire kit shelf life. It is allowed to freezing/thaw up to 5 times. After opening, store under the same conditions as the reagents before opening.

A reagent kit stored in violation of the regulated regime cannot be used.

Transportation

Cito-CoV-2-Test reagent kit should be transported by all types of covered vehicles in accordance with the transportation rules applicable to this transport type.

Cito-CoV-2-Test reagent kit should be transported at temperatures from -16 to -24°C during the entire kit shelf life. It can be transported at 4°C for up to three days.

Atmospheric pressure is not subject to control, as it does not affect the product quality.

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 ice packs. The type, volume and quantity of ice packs placed in a thermal container with transported reagent kits, as well as the thermal container volume are selected depending on the transportation duration and conditions.

Attention! Ice packs thawing during transportation is not a sign of transportation conditions violation, as it is allowed to transport the kit at 4°C up to three days.

Reagent kits transported in violation of the temperature regime cannot be used.

The shelf life of Cito-CoV-2-Test reagent kit is 9 months from the acceptance date of the manufacturer's QCD, if all transportation, storage and usage conditions are met. A reagent kit with an expired shelf life cannot be used.

Shelf life of the opened reagent kit Cito-CoV-2-Test **components** – 9 months from the acceptance date of the manufacturer's QCD, if stored at temperatures from -16 to -24°C.

Shelf life of the kit components prepared for work

One hour under conditions that prevent the components from drying out, as well as extraneous biological material contamination.

12. Disposal

Reagent kits that have become unusable, including due to expiration date, must be disposed in accordance with the requirements of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial facilities public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures".

The consumer packaging and reagent tubes used are not related to human biological fluids and, in accordance with the classification of medical waste, belong to Class A (epidemiologically safe waste, close in composition to solid household waste). Unused reagents in accordance with part X, clause 170 SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, for water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization

and conduct of sanitary and anti-epidemic (preventive) measures" are collected in a reusable labeled packaging in any color (except yellow and red).

The remaining tubes and materials after the work completion are disposed of in accordance with MU 287-113 "Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices".

Liquid components (reagents) are destroyed by draining into the sewer with preliminary dilution of the reagent with tap water 1:100 and removal of remaining packaging as industrial or household waste.

Cito-CoV-2-Test reagent kit consumer packaging is subject to mechanical destruction with removal of residues as industrial or household waste.

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

13. Warranty, contacts

The manufacturer guarantees Cito-CoV-2-Test reagent kit compliance with TS requirements if all transportation, storage and usage established requirements are met.

If you have any complaints about the kit quality, please contact:

Limited Liability Company TestGene (TestGene LLC),
9, 44th Inzhenerny Proezd, office 13, Ulyanovsk, 432072
Phone number: +7 (499) 705-03-75

www.testgene.com

Technical Support Service:

Phone number: +7 927 981 58 81

E-mail: help@testgen.ru

The instructions for use comply with the requirements of the Order of the Ministry of Health of the Russian Federation dated 09.01.2014 No. 2n, Order of the Ministry of Health of the Russian Federation dated 19.01.2017 No. 11n, GOST 51088-2013.

Annex A

GOST ISO 14971-2011	Medical devices. Application of risk management to medical devices.
GOST R EN 13612-2010	Performance evaluation of <i>in vitro</i> diagnostic medical devices
GOST R 56894-2016	Summary technical documentation for demonstrating conformity to the essential principles of safety and performance of <i>in vitro</i> diagnostic medical devices
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.
GOST R ISO 23640-2015	<i>In vitro</i> medical devices. Evaluation of stability of <i>in vitro</i> diagnostic reagents.
GOST R 51088-2013	<i>In vitro</i> diagnostic medical devices. Reagents, kits, test-systems, control materials, culture media. Requirements to devices and to supporting documentation.
GOST R ISO 15223-1-2020	Medical devices. Symbols to be used with medical device labels, labelling and information to be supplied. Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-1-2024	<i>In vitro</i> diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2024	<i>In vitro</i> diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 2. <i>In vitro</i> diagnostic reagents for professional use.
GOST R 51352-2013	<i>In vitro</i> diagnostic medical devices. Test methods.

Note – The above standards were in force at the time of the instructions for use approval. In the future, when using the document, it is advisable to check the validity of the reference normative documents at the current moment. If the reference document is replaced or modified, then the replaced (modified) document should be used when applying this document.