Approved
Syntol LLC, CEO

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INSTRUCTION

For medical use of the reagent kit for detection of coronavirus SARS-CoV-2 RNA by real-time polymerase chain reaction (OM-Screen-2019-nCoV-RT)

TU 20. 59.52- 032 - 46395995 -2020

2020

1 PURPOSE

1.1 The full name of the reagent kit

"The reagent kit for the detection of RNA of coronavirus SARS-CoV-2 by Real-Time polymerase chain reaction (OM-Screen-2019-nCoV-RT)", TU 20.59.52-032-46395995-2020.

1.2 Purpose of the kit and its diagnostic function

The kit "OM-Screen-2019-nCoV-RT" is intended for the qualitative detection (screening, monitoring) of coronavirus SARS-CoV-2 RNA in nucleic acid (NA) samples extracted from biological materials, such as nasal, nasopharyngeal or oropharyngeal smear, bronchial washings obtained by fibrobronchoscopy (bronchoalveolar lavage), (endo) tracheal, nasopharyngeal aspirate, sputum, biopsy or autopsy lung material, whole blood, serum, urine.

The diagnostic function of the kit is to support the diagnosis of specific disease (the severe acute respiratory infection - COVID-19) by analyzing samples obtained from a patient (suspected of disease) and specific detection of the pathogenic biological agent (PBA).

1.3 Field of application

Clinical laboratory diagnostics; severe acute respiratory infection – COVID-19; PBA specific detection. The kit can be used by medical institutions and public health authorities.

1.4 Directions

Identification of SARS-CoV-2 RNA by real-time PCR should be performed for the patients with clinical symptoms of respiratory disease and suspected of infection caused by SARS-CoV-2, particularly coming from the epidemiologically unsafe regions immediately after the initial inspection, and contact persons, according to “Provisional guidelines for prevention, diagnosis and treatment of the novel coronavirus infection (2019-nCoV)”, approved by Ministry of Health of Russian Federation and Rospotrebnadzor (Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing).

2 KIT CHARACTERISTICS

Kit components are single-use.

Reagent kit "OM-Screen-2019-nCoV-RT" does not require maintenance or calibration.

2.1 Composition of the kit

The reagent kit consists of 5 reagents.

#	Kit components	Description	Volume	Qty
1.	The lyophilized reaction mixture in stripped microtubes, **PC–nCoV**	white dry substance	-	6 strips of 8 microtubes
2.	Diluent, **RB**	clear colorless liquid	0.8 ml	1 test tube
3.	The lyophilized positive control sample, **PCS-nCoV**	white dry substance	-	3 test tubes
4.	Solution for diluting PCS, negative control sample, **NCS**	clear colorless liquid	1 ml	1 test tube
5.	Internal positive control of extraction, **IPC-Extr-RNA**	clear colorless liquid	0.5 ml	1 test tube

Note: The ingredients that may affect the assay results are primers and fluorescent probes, whose basic characteristic for the end user is the annealing temperature Ta – 58°C. This characteristic determines the temperature-time regime of amplification; therefore, it should be strictly observed.

2.2 Number of samples tested

The reagent kit is designed for 48 tests, including control samples.

2.3 Method of testing

Detection of nucleic acid fragments of coronavirus SARS-CoV-2 is based on the method of **ONE-STEP SINGLE-TUBE REVERSE TRANSCRIPTION REACTION COMBINED WITH REAL-TIME POLYMERASE CHAIN REACTION (qRT-PCR).**

For amplification by qRT-PCR two oligonucleotide primers flanking the *orf1ab* gene fragment of the coronavirus SARS-CoV-2 genome, two oligonucleotide primers flanking the sequence of qRT-PCR internal positive control (an artificial sequence not

present in nature), two oligonucleotide primers flanking the sequence of the internal positive control of extraction (IPC-Extr-RNA, another artificial sequence not present in nature), as well as three fluorescent probes (labeled by FAM, R6G, and Cy5 fluorescent dyes) are used. Amplification of the specific fragments includes repeated cycles of DNA thermal denaturation followed by annealing of primers to complementary sequences, and subsequent extension of the polynucleotide chains of these primers by DNA polymerase with fluorescent detection of the products in real time. The qRT-PCR is carried out by real-time PCR instruments, such as ANK-32 (32M, 48) (Syntol, Russia), CFX-96 (BioRad, USA), Rotorgene, or other equipment equivalent in characteristics (see Item 5.1 of this Instruction).

The kit takes advantage of the multiplex qRT-PCR in terms of simultaneous detection in a single tube of coronavirus SARS-CoV-2 RNA, as well as **two internal controls**. Fluorescence signals are recorded independently in the following channels:

- **FAM -** internal positive control of RNA extraction quality;
- **R6G** - coronavirus SARS-CoV-2 RNA;
- **Cy5** - internal positive control of qRT-PCR quality.

A positive test result means that the test sample/material contains coronavirus SARS-CoV-2 RNA. A negative result means that the test sample/material does not contain SARS-CoV-2 viral RNA.

2.4 Limitations of the Method

For RNA extraction, reagent kits with known characteristics related to possible interfering substances are used ("M-Sorb-OOM", SYNTOL, Cat. N. OOM-502).

The reason of obtaining a false positive result may be the contamination at the step of RNA extraction or during preparation of the qRT-PCR reaction. False positive results are detected by negative control samples added at the steps of extraction and qRT-PCR (Item. 8.3 of this Instruction).

The reason of obtaining false negative result is inhibition of qRT-PCR and/or insufficient quality of RNA extraction. False negative results are detected by internal positive control samples added during RNA extraction (IPS-Ex-RNA).

3 ANALYTICAL AND DIAGNOSTIC CHARACTERISTICS

3.1 Analytical characteristics

3.1.1 Sensitivity

Sensitivity is **1000 copies of RNA per ml** of sample (copy·ml^{-1}). To achieve this sensitivity, it is recommended that kits used for RNA extraction allow the use of aliquots of the tested material (sample) with a volume of at least 100 μl.

3.1.2 Specificity

False-positive results were not obtained when assaying samples of the following respiratory infection pathogens: viruses of Severe Acute Respiratory Syndrome (SARS-CoV), the Middle East Respiratory Syndrome (MERS-CoV), influenza A H1N1, H5N1 and B, respiratory syncytial virus (RSV), adenoviruses, *Coxiella burnetii*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type B, *Legionella pneumophila*, Metapneumovirus, Human respirovirus 1, Rhinovirus (14 negative samples).

In the studies, 100% reproducibility was demonstrated for all positive samples in setting, between sets and between series.

In case of doubt regarding the validity of a positive test result, the functional characteristics (detection ability) of the “OM-Screen-2019-nCoV-RT” kit are to be checked using the DNA sequencing method. For this, the amplification product should be obtained from the sample with questionable result using oligonucleotide primers specific for the fragment of *orf1ab* gene from coronavirus SARS CoV-2 genome with nucleotide positions 5327-5515 of the reference sequence NC_045512.2 (Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome). Primers are available on request from the manufacturer:

SYNTOL LLC (127550, Russia Moscow, Timiryazevskaya Str., 42, Building B, room 316. Phone +7 (495) 984-69-93, FAX +7 (499) 977-74-55, E-mail: syntol@syntol.ru).

The obtained PCR product should be sequenced by the Sanger method.

3.2 Diagnostic characteristics

Clinical studies of the reagent set kit have been successfully performed by State Research Center of Virology and Biotechnology VECTOR (R.S. Koltsovo, Novosibirsk region).

4 SAFETY PRECAUTIONS

Operations with material infected or suspected of to be infected with the coronavirus SARS-CoV-2 should be carried out in accordance with the sanitary-epidemiological rules for the safety of operation with microorganisms of class I-II biological hazard (SP 1.3.3118-13), guidelines "Organization of Laboratories that use NA amplification methods operating with material containing microorganisms of class I-IV biological hazard" (MU1.3.256909), provisional methodical recommendations “Prevention, diagnosis and treatment of the novel coronavirus infection (2019-nCoV)” (Provisional Methodological Recommendations 2019-nCoV), and “Provisional methodical recommendations for the laboratory testing and diagnostics of the novel coronavirus 2019 (2019-nCoV) by polymerase chain reaction (PCR)”.

At the same time, it is necessary for personnel to ensure and comply with the biological safety rules and the requirements to prevent contamination by nucleic acids and (or) PCR products of the studied samples, laboratory area and equipment.

The potential risk of using the kit is class 3 (clause 9 of annex 2 to the order of the Ministry of Health of Russia dated 06.06.2012 No.4n).

4.1 Required staff qualification

The reagent kit should be used by a qualified specialist with higher medical education, PCR diagnostics training, and with a license to work with class I-II biological hazard PBA, assisted by a laboratory technician with special medical education.

4.2 Safety measures to protect the operator

When working with the kit, please comply with the requirements of GOST R 52905-2007 “Medical laboratories. Safety requirements" standard. All components of the kit in the concentrations used are non-toxic, they do not have a harmful effect on the

operator's body. When working with the kit, the usual laboratory precautions should be followed:

- use laboratory gloves and wear a laboratory coat;
- do not eat, drink or smoke in the laboratory;
- after working with samples and reagents, you should thoroughly wash your hands with water and soap.

Avoid reagent contact with skin, eyes and mucous membranes. If they are contaminated with kit components wash with plenty of water. If reagents are ingested, it is necessary to contact immediately for medical assistance.

4.3 General safety measures against physical factors

When in use, there is no need to take precautions against the influence of magnetic fields, external electrical effects, electrostatic discharge, pressure or pressure drops, overload, thermal sources of ignition.

4.4 Safety measures against specific risks

When using the reagent kit, it is not necessary to take precautions against any specific risk in the use or implementation, because the product does not contain substances of human or animal origin, taking into account their potential infectious nature.

5 EQUIPMENT AND MATERIALS REQUIRED WHEN OPERATING THE KIT

5.1 Recommended measuring equipment

The real-time PCR machine equipped for detection channels corresponding to FAM, R6G, ROX, Cy5 fluorescent dyes (or their spectral analogues), such as ANK32, ANK48, DT-Light/DT-Prime, CFX-96, Rotorgene, Light Cycler 480, etc.

5.2 Advice on the use of special equipment

Operation with the kit should be carried out in a desktop cabinet with a bactericidal lamp (for example, BAS-PCR-“Laminar-S”, CJSC “Laminar Systems”, Miass, Russia) installed in the working area class 3 (MU 1.3.2569-09).

5.3 Dosing devices

A set of variable volume pipettes for 0.5-10 μl, 2-20 μl, 10-100 μl, and 20-200 μl.

5.4 Other equipment

Microcentrifuge-vortex (e.g., "Cyclotemp-901");

Microcentrifuge for strips (e.g., "Cyclotemp-903");

Rack, type "IsoFreeze";

Racks for pipette tips, 0.5 and 1.5 ml microtubes;

Racks for working with strips (Syntol LLC);

Refrigerator for 2-8°C.

5.5 Laboratory glassware

Containers for dispensing tips and microtubes.

5.6 Materials and reagents not included in the kit

Disposable tips for variable volume pipettes with an aerosol barrier up to 10 μl;

Disposable tips for variable volume pipettes with an aerosol barrier up to 20 μl;

Disposable tips for variable volume pipettes with an aerosol barrier up to 200 μl;

Reagent kit for DNA/RNA extraction (e.g., "M-Sorb-OOM", Syntol Co., Cat.No. OOM-502);

Individual lab coat and disposable medical gloves;

A set of tools for cleaning the workplace.

6 ANALYZED SAMPLES

6.1 Type of test samples

Biologic materials for study are the following: samples produced by taking a nasal, nasopharyngeal, and/or oropharyngeal smear, the washes obtained by fibrobronhoscopy (bronhoalveolar lavage), (endo)tracheal, nasopharyngeal aspirate, sputum, biopsy or autopsy material of the lungs, whole blood, serum, urine.

The main sample type for the assay is supposed to be a swab from nasopharynx and/or oropharynx.

6.2 Procedure for obtaining biological material

To perform the assay, sampling is carried out in accordance with the methodical recommendations “Prevention, diagnosis and treatment of the novel coronavirus infections (2019-nCoV)” (Provisional MR 2019-nCoV).

6.3 Limitations on the use of analyzed material

To extract RNA, a special reagent kit is used (for example, "M-Sorb-OOM", Syntol Co., Cat.No. OOM-502). For required sensitivity, it is recommended to use kits for DNA/RNA extraction that allow extraction from the sample volume not less than 100 μl. The resulting RNA sample is assayed using the OM-Screen-2019-nCoV-RT reagent kit.

In addition to the analyzed sample, it is necessary to include a negative control (**NIC-B**) in each procedure of extraction, which is then tested by qRT-PCR. This allows to control possible contamination at the stage of NA extraction.

For control of nucleic acids extraction, use the tube **IPC-Extr-RNA** from the kit "OM-2019-Screen-nCoV-RT". 10 μl of **IPC-Extr-RNA** is added to the sample tube immediately after the lysis step. Detection of PCR signal growth in the **FAM** channel indicates successful RNA extraction.

6.4 Conditions of transportation and storage of the analyzed samples

Transportation of biological material to the laboratory and storage prior to the assay should be carried out according the requirements of the provisional methodical recommendations "Prevention, diagnosis and treatment of the novel coronavirus infection (2019-nCoV)» (Temporary MP 2019-NcoV) and "Provisional methodical recommendations for the laboratory diagnostics of the novel coronavirus 2019 (2019-nCoV) by polymerase chain reaction (PCR)".

6.5 Precautions for study material

Registration, storage, transfer and transport of the biological material suspected of the presence of severe acute respiratory infection COVID-19 should be carried out in accordance with the sanitary and epidemiological safety rules for operation with microorganisms of class I-II biological hazard (SR 1.3.3118-13) and current sanitary rules on the registration, storage, transfer and transportation of microorganisms of class I-II biological hazard.

Clinical material is disposed by class B as extremely epidemiologically hazardous waste in accordance with the sanitary epidemiology requirements for the treatment of medical waste (Sanitary rules and norms, RF 2.1.7.2790-10).

7 PREPARATION OF REAGENTS FOR TESTING

Reaction mixture in stripped microtubes: 15μl of RB (DB, diluent) should be added into tubes with dry **PC-nCoV**. The prepared solution should be used immediately in the current test.

Diluent RB: ready to use.

Positive control sample, PCS-nCoV: 200μl of **NCS** is added into a vial with dry **PCS-nCoV**, then mixed by vortexing until complete dissolution, centrifuged for 5 seconds at 3000 rpm. Storage of diluted PCS-nCoV is allowed frozen at a temperature below -20°C during the shelf life of the kit.

Solution for diluting PCS, NCS: ready to use.

Internal positive control of extraction, IPS-Extr-RNA: ready to use.

8 ASSAY OPERATION

The assay is performed in the working area 3 free of contact with biological materials (MI 1.3.2569-09).

8.1 Minimal amount of each reagent required for the assay

For one test, the following reagent amounts are consumed:

- one microtube with **PC-nCoV**;
- 15 μl of **DB**.

For each test run, the following reagent amounts are consumed:

- 20 μl of **PCS-nCoV**;
- 20 μl of **NCS**.

8.2 Assay steps

The assay using the kit is carried out in one step:

- turn on the real-time PCR instrument, start a software according with the operation manual;

- enter the parameters of temperature-time regimen of amplification according to Table 2 and the following dyes: **FAM**, **R6G/HEX** and **Cy5**;

Table 2 - Temperature-time mode of amplification

No.	Temperature-time mode	Number of cycles
1	50ºC – 900 seconds	1
2	95ºC - 300 seconds	1
3	95ºC – 10 seconds	50
4	58°C – 10 seconds **Fluorescence reading**	
5	72ºC – 20 seconds	

- enter sample information for all dyes; start the real-time PCR instrument operation in accordance with the operation manual. For RotorGene instrument, the following level values should be set up: Green – 4, Yellow – 8, Red – 8.

NOTE: When working with RNA, it is necessary to use only disposable plastic consumables with a special marking "RNase-free", "Dnase-free"!

- take the kit from refrigerator; vortex **DB** and **NCS** and centrifuge for 5 sec at 3000 rpm;
- place stripped microtubes (**PC-nCoV**) in a rack for strips type "IsoFreeze"* and mark in accordance with the testing protocol;
- open caps of the microtubes and add 15 μl of **DB into each tube**;
- using tips with aerosol barrier, add into microtubes solutions in the following order:
    - first, add 20 μl **NCS** and close the microtube cap;
    - next, add 20 μl of test samples in the required replicate number, then close the microtube caps;
    - last, add 20 μl of **PCS-nCoV** to the microtubes according to the marks, and close the microtube caps;
- mix the content of the microtubes by vortexing and centrifuge for 5 sec at 3000 rpm.

ATTENTION! **After mixing PC-nCoV, diluent and RNA sample, microtubes should be immediately installed into a real-time PCR instrument, and the cycling protocol should be started!**

- place microtubes in the real-time PCR instrument according to the study protocol and check that all caps are properly closed;
- start the qRT-PCR reaction and write down the file name in the workbook.

Approximate qRT-PCR run time is about 1.5 hour.

8.3 Procedure for measuring and evaluating test results

Evaluation of the results is carried out using real-time instrument software (see Item 5.1 of this Instruction).

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* Note: Prior to operation, it is recommended to cool a rack in a refrigerator at the temperature of 2-8 °C.

** Note: each sample is recommended to study **in duplicate**.

The positive fluorescence signal growth (registering growth of the signal, Fig.1) refers to any value of the threshold cycle (Ct) less than 50. The absence of signal growth is described by instrument program, as a value of the threshold cycle (Ct >= 50 in ANK instruments, Syntol) or no amplification (N/A in CFX96 instruments, BioRad, etc.). Detailed information is provided by the instruction manual for real-time PCR instrument.

The positive fluorescence signal growth in the channel **R6G/HEX** (green color curve) (Fig.1) compared to no growth for the negative control sample (Fig.2) indicates the presence of specific fragments of coronavirus SARS-CoV-2 RNA in tested sample.

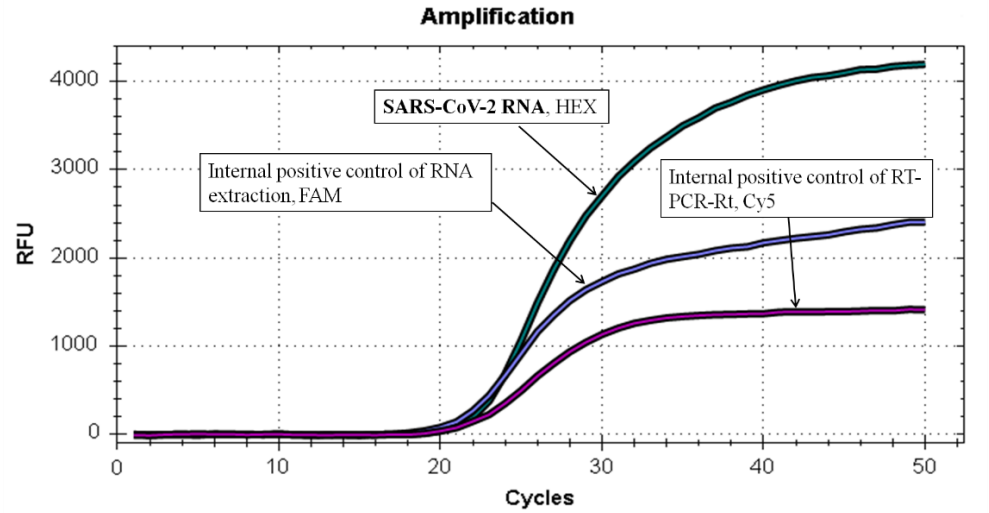

Fig.1 Example of SARS-CoV RNA positive test sample.

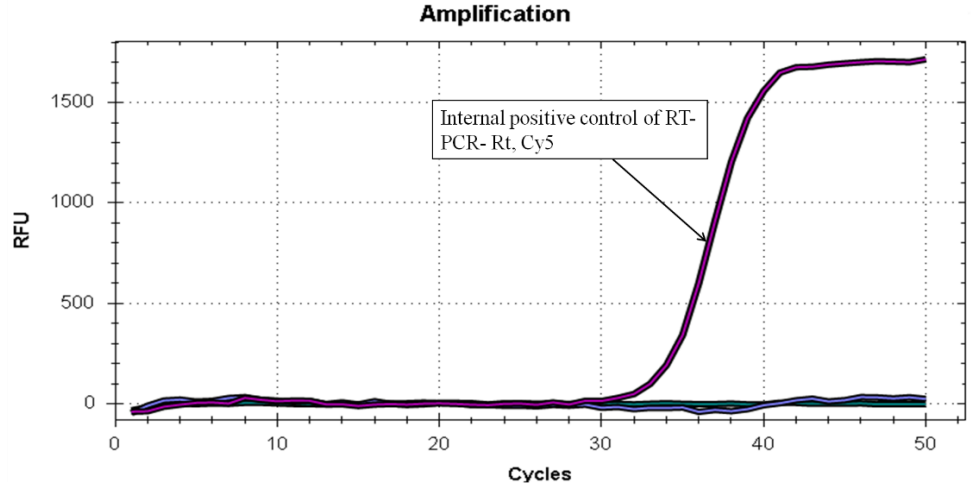

Fig. 2 Correct Negative Control Sample (NCS) amplification result.

The fluorescence signal growth in channel **Cy5** (purple color curve) in the case of the absence of positive growth in the channel **R6G/HEX** indicates successful completion

of reverse transcription (RT) and real-time PCR and confirms the absence of specific fragments of coronavirus SARS-CoV-2 RNA in a sample (Fig.3).

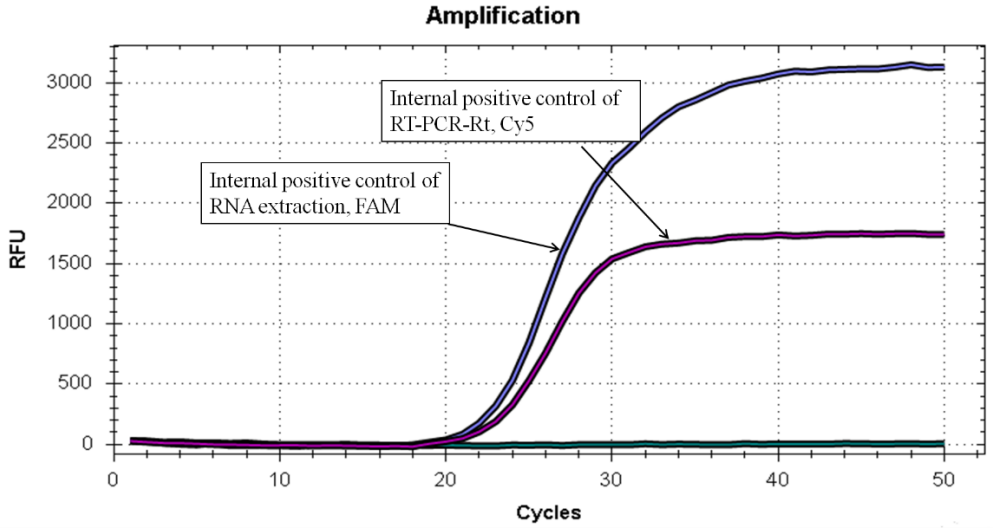

Fig.3 Example of SARS-CoV RNA negative test sample.

The growth of the fluorescence signal in channel **FAM** (blue color curve) in the absence of positive dynamic in channel **R6G/HEX** indicates successful extraction of RNA from the sample and confirms the absence of specific fragments of SARS-CoV-2 RNA in the tested sample (Fig.3). The example of Positive Control Sample amplification is shown in Fig.4.

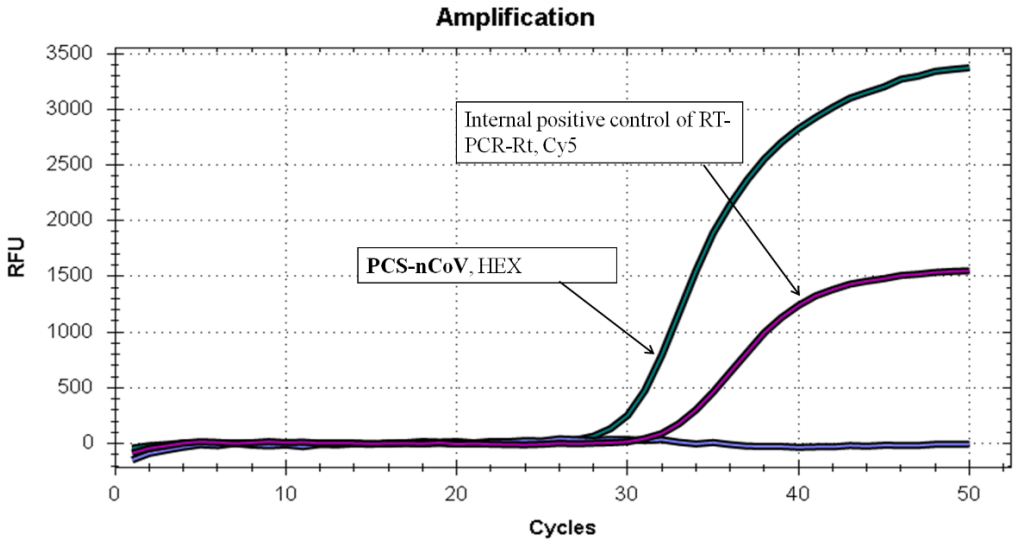

Fig. 4 Correct Positive Control Sample (PCS-nCoV) amplification result.

Registration of test results

Test results are not valid:

- in the case of detecting the growth of signal in the channel **R6G/HEX** for the **NCS** sample (false-positive result of the entire run). The value of the threshold cycle Ct in this case does not matter;
- in the absence of the growth of **PCS-nCoV** signal in the channel **R6G/HEX** (false-negative result of the entire run);
- in the absence of the growth of signal in the channel **Cy5***** (false-negative result in a particular microtube).

Results of the analysis are valid:

- in the absence of growth of signal in the channel **R6G/HEX** for **NCS** sample;
- in the case of detecting the growth of signal in the channel **Cy5***,** with any value of Ct threshold cycle;
- in the case of detecting the growth of **PCS-nCoV** signal in the channel **R6G/HEX**, with any value of Ct threshold cycle.

Registration of signal growth in the **R6G/HEX** channel in microtubes with tested NA samples/material indicates the presence of specific RNA fragments of the coronavirus SARS-CoV-2 genome in the sample. The value of the threshold Ct cycle in this case does not matter.

Registration of signal growth in the **Cy5** (Red)*** channel with a threshold Ct cycle less than 37.0 indicates the successful reverse transcription and real-time PCR in each particular microtube, higher Ct values indicate some inhibition of qRT-PCR.

*** Note: In the microtubes with a signal growth in the **R6G/HEX** channel with a threshold cycle (Ct) of less than 30.0, it is possible that the signal growth is not recorded or amplitude of the kinetic curve is low on the **Cy5** (Red) and **FAM** (Green) channels.

If **IPC-Extr-RNA** sample was added at the stage of RNA extraction, detection of the signal growth in the **FAM** channel with a threshold cycle of less than 35.0 indicates successful RNA extraction from the sample, larger Ct values indicate some RNA loss at the stage of RNA extraction.

The interpretation of the analysis results is carried out in accordance with Table 3.

Table 3 - Interpretation of the assay results

The qRT-PCR result by fluorescence channel			Sample	Interpretation
Fam	**R6G/HEX**	**Cy5**		
The **whole** assay result is **not valid** in the following cases				
-	-	-	**PCS**	False negative result, inhibition of qRT-PCR
any	+	any	**NCS-Extr**	False positive result, contamination in the process of RNA extraction (in case when NCS was added before RNA extraction)
	+		**NCS**	False positive result, contamination during preparation of qRT-PCR
The test results is **not valid** for **particular** sample in the following cases				
-	-	-	**Test sample**	False negative, inhibition of qRT-PCR
- *	-	+		False negative result, insufficient efficacy of RNA extraction
The test results **are valid** in the following cases				
-	+	any	**PCS**	Reagent kit is specific for RNA of coronavirus SARS CoV-2
+ *	-	+	**ICS-Extr**	No contamination
-	-	+	**ICS**	
+ *	-	+	**Test sample**	**The sample is negative** (no coronavirus SARS-CoV-2 RNA was detected in the sample)
any	+	any		**The sample is positive** (sample contains coronavirus SARS-CoV-2 RNA)
* Note: in the case when IPC-Extr-RNA was added at the stage of RNA extraction				

9 CALCULATIONS

To carry out qRT-PCR and evaluate its results, the special software provided by the manufacturer of the PCR instrument should be used, such as “ANK_Shell” version 100 and higher supplied with “ANK-32M”, “ANK-48”, or CFX Manager 2.0 and higher supplied with Bio-Rad CFX-96 devices. Operation with the software is carried out in accordance with the instrument’s operating manual.

10 STORAGE, TRANSPORTATION AND OPERATION CONDITIONS FOR THE KIT

10.1 Storage conditions

The kit should be stored in a dry place protected from light at a temperature from +2 to +8°C. The total storage at temperatures from +9 to +20°C should not exceed 14 days within the period of the kit shelf life.

10.2 Transportation conditions

The transportation of the “OM-Screen-2019-nCoV-Rt” reagent kit should be carried out according TU 20.59.52-032-46395995-2020 by covered vehicle (by road, rail, or air) at temperatures from +2 to +8 °C. Transportation up to 14 days is allowed at temperatures up to +20 °C.

10.3 Shelf-life of the kit

The shelf life of the kit is 12 months from the date of manufacturing. Expired reagent kits cannot be used. The expiration date of the opened kit corresponds to the expiration date indicated on the labels of unopened reagents, unless otherwise specified in the instruction.

10.4 Information on safe disposal

Used tubes with PCR products, tips, gloves, rags for surface treatment in a PCR box should be collected in plastic lockable containers, taken out into the working area 4-1 or specially intended rooms (MU 1.3.2569-09) for the purpose of subsequent inactivation according to the requirements of SanPiN 2.1.7.2790-10.

Kits with expired shelf life, as well as in case of packaging damage, are disposed by class A as an epidemically low hazard waste, similar in composition to MSW (SanPiN 2.1.7.2790-10).

10.5 Warranty of the manufacturer

The manufacturer guarantees the compliance of the functional characteristics of the reagent kit with the specifications of the technical and operational documentation for a specified shelf life (12 months), subject to all conditions of transportation, storage and use. Kit quality claims should be directed to the manufacturer: Syntol LLC (127550, Moscow, 42 Timiryazevskaya St., building B, room 316, tel. (495) 984-69-93, fax. (499) 977-74-55 E.mail: syntol@syntol.ru).

In case of side effects not specified in the instructions for the use of the reagent kit, undesirable reactions when using it, facts and circumstances that threaten the life and health of citizens and medical workers when operating the reagent kit, it is recommended to send a report to the manufacturer, Syntol LLC by the address provided above and to an authorized state regulatory organization in accordance with the applicable law.