For professional use only

eDetect SARS-CoV-2/SARS-CoV Multiplex Kit USER MANUAL

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TABLE OF CONTENTS

1.	INTENDED USE	3
2.	METHOD	3
3.	CONTENT	4
4.	REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED	4
5.	TRANSPORT AND STORAGE CONDITIONS	6
6.	WARNINGS AND PRECAUTIONS	6
7.	SAMPLES	8
8.	PROCEDURE	9
9.	CONTROLS	12
10.	DATA ANALYSIS	13
11.	SPECIFICATIONS	15
12.	TROUBLESHOOTING	16
13.	QUALITY CONTROL	17
14.	KEY TO SYMBOLS	18

1. INTENDED USE

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** is intended for research and diagnostic applications. The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit i**s an *in vitro N*ucleic Acid Test (NAT) – pathogen-detection-based product. The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit i**s designed to detect SARS-CoV-2 (COVID-19 virus, 2019-nCoV) and SARS-like coronaviruses in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm.

Indications for the use:

- persons with ARVI symptoms and who have been in contact with COVID-19 infected, regardless of their age;
- persons of all ages without ARVI symptoms (in the centers of infection/ in the conditions of infection spread) for the purpose of early detection of coronavirus to prevent further spread of infection.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use the **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit.**

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice. The safety of laboratories should be ensured in accordance with the requirements of legislation in the field of sanitary and epidemiological welfare.

Potential users: personnel qualified in molecular diagnostics methods and in working with pathogenic microorganisms and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this user manual.

2. METHOD

The implemented method of reverse transcription followed by polymerase chain reaction is based on RNA reverse transcription process and subsequent amplification of cDNA.

The RNA reverse transcription stage and PCR amplification of cDNA stage are performed in one test tube.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA during the initial heating of the tube.

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** includes the Internal control (RNA-IC “A”), which is intended to assess the quality of the RNA extraction and polymerase chain reaction.

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains four target-specific probes bearing reporter fluorescent dyes (Fam, Hex, Rox and Cy5) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

DNA probe used for the detection of the SARS-CoV-like coronaviruses product amplification includes fluorescent dye Fam. DNA probe used for the detection of the SARS-CoV-2 (E-gene) product amplification includes fluorescent dye Rox. DNA probe used for the detection of the SARS-CoV-2 (N-gene) product amplification includes fluorescent dye Cy5. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of four fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red
SARS-CoV-like Coronaviruses	IC*	SARS-CoV-2 coronavirus, E-gene	SARS-CoV-2 coronavirus, N-gene

* Internal control (RNA-IC "A")

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** is also approved for use with Rotor-Gene Q (Qiagen) and CFX96 (Bio-Rad) real-time thermal cyclers.

3. CONTENT

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** content is represented in Table 2.

Table 2. The **SARS-CoV-2/SARS-CoV Multiplex Kit** content for ED1003

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	1440 µL (15 µL per tube)	12 8-tube strips
RT-PCR-buffer	Colorless transparent liquid	1620 µL (810 µL per tube)	2 tubes
Enzyme Taq/RT	Colorless transparent viscous liquid	55 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL per tube)	2 tubes
Internal control (RNA-IC "A")	Colorless transparent liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps	12 8-caps		

All components are ready to use and do not require additional preparation for operation.

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

It is not recommended to perform less than 8 samples (6 defined samples, one positive control and one negative control) in one run. It can lead to situation when the volume of enzyme will be insufficient.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single use sterile containers to collect clinical material;

4.2. RNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II-III;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF 12000 – 16000 x g);
- Solid-state thermostat (temperature range 40-95°C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL microcentrifuge tubes with caps;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free filtered pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 0.2-1000 µL volume range);
- RNase and DNase free filtered pipette tips for semi-automatic pipettes (volume 20 µL, 200 µL, 1000 µL);
- Nucleic acid extraction kit. Any commercial RNA/DNA isolation kit can be used if it is CE-IVD validated for the sample types. Ecoli Dx, Ltd. recommends the use of the ePure Viral Nucleic Acid Extraction Kit E2003;
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes or strips;
- Rotor for strips (if package in strips is used);
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit**, except Enzyme Taq/RT, must be stored at temperatures from 2 °C to 8 °C during the storage period. The PCR-mix for amplification must be stored out of light at temperatures from 2 °C to 8 °C during the storage period. The excessive temperature and light can be detrimental to product performance. The Enzyme Taq/RT must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the Enzyme Taq/RT, is allowed in termobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the Enzyme Taq/RT in termobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- Enzyme Taq/RT should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kits stored under undue conditions should not be used.

An expired **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit**

6. WARNINGS AND PRECAUTIONS

The SARS-CoV-2 coronavirus is classified as particularly pathogenic. Laboratories performing research on the detection of SARS-CoV-2 RNA are required to ensure the safety of work in accordance with the requirements of national legislation in the field of sanitary and epidemiological welfare.

Only specially trained personnel with medical or biological (veterinary) education who have been trained at licensed courses of primary specialization in working with pathogenic microorganisms and who have received additional special training at advanced training courses on molecular and biological methods of diagnostics are allowed to work with the kit of reagents.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a

way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA**.** Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Use powder-free surgical gloves and protective clothing (work clothes and personal protective equipment). Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121ºC before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and cannot be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a special closed container containing a disinfectant solution. Do not open the tubes after amplification. Work surfaces, as well as rooms where PCR is performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breach;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** is designed to detect RNA extracted from the nasopharynx and oropharynx swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm, depending on professional prescription.

Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors are the presence of hemoglobin in the RNA sample as a result of incomplete removal during the extraction of RNA from a biomaterial sample containing an impurity of blood, as well as the presence of isopropyl alcohol and methyl acetate in the RNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: hemoglobin – 0.35 mg/mL cDNA sample, isopropyl alcohol – 100 µL/mL cDNA sample, methyl acetate – 100 µL/mL cDNA sample.

Impurities contained in the biomaterial sample, such as mucus, blood, elements of tissue breakdown and inflammation, local medicines, including those that are contained in nasal sprays, etc. should be removed during the NA extraction using sample preparation kits. To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose NA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of NA extraction.

The features of biomaterial sampling

Work with biomaterials should be performed in accordance with Laboratory testing for coronavirus disease (COVID-19) in suspected human cases, Interim guidance, 19 March 2020 and national legislation.

The collection of clinical material and its packaging is carried out by an employee of a medical organization who is trained in the requirements and rules of biological safety when working and collecting material suspected of being infected with pathogenic microorganisms.

The timing of biomaterial sampling is very important. Presumably, the highest content of the virus in the respiratory organs of person can be within the first 4 days after the appearance of symptoms of the disease. Samples should be collected within 3 days after the appearance of clinical symptoms of the disease.

At least three types of clinical material should be collected from one patient.

It is necessary to take swabs from the nasal cavity, naso-and oropharynx.

Each sample of biomaterial should be placed in a separate transport container.

Transportation and storage of the samples

Type of the sample	Collecting material requirements	Transportation	Storage conditions before transportation	Comments
Nasopharynx and oropharynx swabs	Plastic test tubes and tampons for swabs**	4 °C	≤5 days: 4 °C >5 days *: -70 °C	Nasopharyngeal and oropharyngeal tampons should be placed in the same tube to increase the viral load
Bronchoalveolar lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: -70 °C	A small sample dilution is possible
Endotracheal aspirate, nasopharyngeal aspirate or nasal lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: -70 °C	
Phlegm	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: -70 °C	Make sure that the material is from the lower respiratory tract

* if it is not possible to store samples at minus 70 °C, store samples at minus 20 °C.

** Use a transport medium for storage and transportation of the respiratory swabs or saline solution (if transportation to the laboratory no more than 24 hours after taking the sample) or a dry probe-tampon (if transportation to the laboratory no more than 4 hours after taking the sample).

It is recommended to use transport media containing preservatives, intended for further study of samples by PCR.

Avoid repeated freezing and thawing of samples.

Samples must be transported in accordance with the requirements of the sanitary legislation in relation to pathogenic microorganisms.

8. PROCEDURE

The range of SARS-CoV-2 viral load can vary widely from very low values (104 or less copies/mL) in the biomaterial of asymptomatic carriers and patients in the recovery stage to extremely high values (more than 109 copies/mL) in the biomaterial of patients with a clinical picture of acute viral pneumonia. In this regard, when performing research in a clinical laboratory, the risk of cross-contamination between samples at all stages of work is a serious danger, especially during aliquoting and RNA extracting. Cross-contamination with high-copy biomaterial can lead to sporadic false-positive results.

To prevent cross-contamination of the biological material in the laboratory, the following rules are recommended:

- it is necessary to conduct a visual assessment of the incoming biomaterial and cull test tubes with broken integrity;
- if possible, it is recommended to analyze samples of patients from a hospital with symptoms of acute infection separately from the rest of the samples (the biological material for screening exposed individuals and patients with mild disease). It is desirable to work with the supposed high-copy samples in a separate box or after working with the supposed low-copy samples;
- It is necessary to use negative control samples, starting from the stage of extracting RNA in each protocol;
- use tips with aerosol filters at all stages of the assay;

- strictly follow the assay procedure, open the Eppendorf test tubes with tweezers (do not touch inside the tube cap by the gloved hand); when applying reagents, do not touch inside the test tube by the tip (if this happened, immediately replace the tip).

8.1 RNA extraction

Any commercial RNA/DNA isolation kit can be used if it is validated by CE IVD for the sample types. Ecoli Dx, s.r.o. recommends the use of ePure Viral NucleicAcid Extraction Kit E2003

RNA extraction is carried out according to the extraction kit instructions.

The volume of the resulting RNA preparation should not exceed 50 µl.

The resulting RNA preparation must be used immediately for RT-PCR. If it is needed, the resulting RNA preparation can be stored at temperatures from minus 18 °C to minus 22 °C for no longer than a week with a single defrost before reverse transcription.

8.2 The features of biomaterial preparation for SARS-CoV-2 coronavirus RNA testing

Do not perform centrifugation as a pretreatment of nasopharyngeal and oropharyngeal swabs (smears) taken into transport medium.

For RNA extraction, 100 µL of the sample is used.

8.3 The use of control samples at the stage of nucleic acid extraction

8.3.1 Internal control sample

To exclude false negative results of the study and to control the quality of the study, it is necessary to use an internal control sample to the clinical samples at the stage of nucleic acid extraction.

The internal control (RNA-IC “A”) from the **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** should be used as an internal control sample. The RNA-IC “A” is an artificial RNA packed in phage particle. It is irrelevant to SARS-CoV-2 and amplified with separate pair of primers and probe.

The RNA-IC “A” should be used in the amount of 10 µL per sample.

The internal control (RNA-IC) and internal control (DNA-IC) from the extraction kit are not used.

8.3.2 Negative control sample

To exclude false positive results of the study and to control the quality of the study, it is necessary to use a negative control sample from the nucleic acid extraction stage.

Independently of DNA/RNA extraction kit used, a negative control sample should go through all stages of DNA/RNA extraction simultaneously with the RNA extraction from clinical samples.

Physiological saline solution can be used as a negative control sample in volumes as indicated in the instructions for use of extraction kits or negative control sample that is include in the corresponding extraction kit

8.4 PCR with Reverse Transcription (RT-PCR)

The reagents and tubes should be kept away from direct sun light.

When using strips, strictly observe the completeness of the strips and caps to them. Do not use the caps to the strips of the other kits!

8.4.1 Mark the required number of the tubes with paraffin sealed PCR-mix according to the number of samples to be analyzed, 1 tube for negative control (C-) and 1 tube for positive control (C+).

Example: to test 6 samples, mark 6 tubes (one for each sample), one for "C-" and one for "C+"). The resulting number of tubes is 8.

8.4.2 Vortex the RT-PCR-buffer and Enzyme Taq/RT thoroughly for 3-5 seconds, then spin briefly for 1-3 seconds.

Enzyme Taq/RT should be got out from the freezer immediately prior to use.

8.4.3 Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT. Add to the one tube:

- 15.0 x (N+1) µL of RT-PCR-buffer;
- 0.5 x (N+1) µL of Enzyme Taq/RT,

N is a quantity of the samples to be tested taking to account "C-", "C+".

Example: to test 6 samples, mark 8 tubes. Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT for 9 (8+1) tubes. Mix 135 µL of RT-PCR-buffer and 4.5 µL of Enzyme Taq/RT.

Taking the Enzyme Taq/RT, it is necessary to dip the tip no more than 1.0 mm and observe the rules for dosing viscous liquids. Thoroughly flush the remaining Enzyme Taq/RT from the tip by pipetting at least 5 times.

8.4.4 Vortex the tube with the mixture of RT-PCR-buffer and Enzyme Taq/RT thoroughly. Then spin briefly for 1-3 seconds.

Mixture of RT-PCR-buffer and Enzyme Taq/RT must be prepared immediately prior to use and should be used within one hour after preparation. If it is needed, the prepared mixture can be stored at the temperatures from 2 °C to 8 °C but for no longer than one hour.

8.4.5 Add 15 µL of the RT-PCR-buffer and Enzyme Taq/RT mixture into each tube. Avoid paraffin layer break.

8.4.6 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close the tubes/strips.

8.4.7 Vortex the tubes with samples and "C-" and "C+" for 3-5 seconds and down the drops for 1-3 seconds.

Open the cap of the tube/strip, add RNA sample (or control sample), then close the tube/strip before proceeding to the next tube/strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.4.8 Add 10 µL of the RNA sample into corresponding tubes. Do not add RNA into the "C-", "C+" tubes. Avoid paraffin layer break.

8.4.9 Add 10 µL of negative control sample (C-), which passed whole RNA extraction procedures into corresponding tube. Add 10 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.4.10 Spin down the tubes for 3–5 seconds to collect drops (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

8.4.11 Set the tubes/strips into the Real-time Thermal cycler.

8.4.12 Conduct RT-PCR taking into attention reaction volume that is 40 µL. See tables 4, 5.

Using the devices of eQuantia: add «SARS2,SARS_RNA-IC» test to the protocol, specify the number and identificators of samples including positive and negative control samples. Define position of tubes in software interface according to position they were set in thermal unit (p. 8.4.11). Run RT-PCR.

Amplification products can be stored at temperatures from 2 °C to 8 °C for one month or at temperatures from minus 20 °C for 12 months.

Table 4. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time, sec	Cycle repeats
Cycling	32 deg	1200	1 time
Cycling 2	95 deg	300	1 time
Cycling 3	94 deg	10	50 times
	60 deg √	15	

√ - optical measurement, set the fluorescence measurement (Acquiring) on the channels Green (Fam), Yellow (Hex), Orange (Rox) and Red (Cy5) at 60 °C

Table 5. The PCR program for CFX96 (Bio-Rad)

Step	Temperature, °C	Time, min:sec	Cycle repeats
1	35	20:00	1
2	95	5:00	1
3	94	0:15	50
4	64 √	0:20	

√ - optical measurement (Plate Read), set the fluorescence measurement on the Fam, Hex, Rox, and Cy5 channels at 64 °C

9. CONTROLS

The **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** contains positive control sample. Positive control is a cloned part of the virus genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The kit includes the Internal control (RNA-IC "A"). RNA-IC "A" is intended to assess the quality of the RNA extraction and polymerase chain reaction. The RNA-IC "A" is an artificial RNA packed in phage particle. It is irrelevant to SARS-CoV-2 and amplified with separate pair of primers and probe. To reveal possible contamination a negative control is required.

A negative control sample should go through all stages of RNA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

The test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;
- the exponential growth of the fluorescence level for the specific product is absence and for internal control is present.

The test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control are not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

10. DATA ANALYSIS

The analysis is based on the presence or absence of specific signal.

The Real-time PCR Thermal Cyclers detects and interprets results automatically. Analysis will be performed by Real-Time PCR application. The interpretation should be performed in accordance with Tables 6, 7.

Table 6. The interpretation of assay results

Detection channel				**Interpretation**
Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red	
Analyzed samples				
Cp/Ct/Cq is specified	Is not considered	**Cp/Ct/Cq is specified**	**Cp/Ct/Cq is specified**	**SARS-CoV-2* RNA is detected**
Cp/Ct/Cq is specified	Is not considered	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	**RNA of SARS-CoV-like coronaviruses is detected**, SARS-CoV-2 RNA is not detected
Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	RNA of SARS-CoV-like coronaviruses is not detected, SARS-CoV-2 RNA is not detected
Positive control sample				
Cp/Ct/Cq is specified	Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	**Cp/Ct/Cq is specified**	Positive result The results are valid
Negative control sample				
Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Negative result The results are valid

*Simultaneous presence of SARS-CoV-2 coronavirus and other coronoviruses like SARS-CoV in the RNA sample is possible

Table 7. Other possible results

Detection channel				**Interpretation**
Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red	
Analyzed samples				
Cp/Ct/Cq ≤ 35	Is not considered	**Cp/Ct/Cq is specified**	Cp/Ct/Cq is not specified	Additional research is required, there is a possible mutation in one of the SARS-CoV-2 genes.
		Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	
Cp/Ct/Cq ≥ 35	Is not considered	**Cp/Ct/Cq ≥ 35**	Cp/Ct/Cq is not specified	**SARS-CoV-2* RNA is detected**
Cp/Ct/Cq is not specified	Is not considered	Cp/Ct/Cq is not specified	**Cp/Ct/Cq ≥ 35**	
Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Unreliable result. Repeat PCR amplification or NA extraction or re-collect of a clinical sample, performed sequentially

*Simultaneous presence of SARS-CoV-2 coronavirus and other coronoviruses like SARS-CoV in the RNA sample is possible

Unreliable results may be caused by the presence of inhibitors in the nucleic acid preparation obtained from the clinical material, errors in the pre-analytical stage, incorrect implementation of the analysis Protocol, non-compliance with the temperature mode of amplification, etc. In this case, either re-staging of reverse transcription and polymerase chain reaction, or re-extracting of the nucleic acid preparation, or re-collect of clinical material (performed sequentially) is required.

When the expressed growing fluorescence (Cp/Ct/Cq is specified) on the Fam/Green, Rox/Orange, or Cy5/Red channels is expressed for negative control (C-), the results of whole series are considered false. It is required to eliminate contamination.

A single negative test result, especially if it is a sample from the upper respiratory tract, does not exclude infection. Lower respiratory tract sampling should be checked for SARS-CoV-2 coronavirus RNA, especially in cases of severe and progressive disease.

Negative results do not eliminate the possibility of SARS-CoV-2 infection and should not be used as the only reason for taking a decision about patient treatment. Negative results should go together with clinical observations and epidemical information.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual).

11. SPECIFICATIONS

a. The analytical **specificity** of the **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

Since it is impossible to exclude the occurrence of new mutations in the genome of the SARS-CoV-2 coronavirus, three genome sites were selected as targets to improve the reliability of diagnostics: the N and E genes sites specific to the SARS-CoV-2 coronavirus, as well as the conservative E-gene site common to the group of SARS-CoV-like coronaviruses (including SARS-CoV and SARS-CoV-2).

In the samples of human biological material with SARS-CoV-2 coronavirus RNA, the detecting amplifier should register an increase in fluorescence on the Fam/Green, Rox/Orange and Cy5/Red detection channels.

In the samples of human biological material free of SARS-CoV-2 coronavirus RNA and SARS-CoV-like coronaviruses RNA, the detecting amplifier should register an increase in fluorescence on the Hex/Yellow detection channel, the increase in fluorescence on the Fam/Green, Rox/Orange, and Cy5/Red channels should be absent.

In the samples of biological material free of SARS-CoV-2 coronavirus RNA, which contains SARS-CoV-like coronaviruses RNA (SARS coronavirus (various isolates); as well as Bat SARS-like coronavirus (various isolates); Bat SARS coronavirus (various isolates); SARS-like coronavirus (various isolates); SARS-related coronavirus (various isolates); Rhinolophus affinis coronavirus; Coronavirus BtRs-BetaCoV), the detecting amplifier should register an increase in fluorescence on the FAM/green detection channel, the increase in fluorescence on the Rox/orange and Cy5/red detection channels should be absent.

There are not non-specific positive results of amplification of RNA sample in the presence of Influenza A virus, Influenza B virus, Human coronavirus HKU-1, Human coronavirus NL-63, Human rhinovirus, DNA of *Mycoplasma pneumonia, Streptococcus pneumonia, Chlamydophila pneumoniae, Haemophilus influenza, Klebsiella pneumoniae, Moraxella catarrhalis, Bordetella pertussis, Bordetella parapertussis*, as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. Analytical sensitivity of the **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit** is **10 copies of RNA** per amplification tube. Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

№ п/п	The concentration of LCS, copies per amplification tube	**Strips**				**Tubes**	
		kit №1		**kit №2**		**kit №1**	
		LCS, series 1	LCS, series 2	LCS, series 1	LCS, series 2	LCS, series 1	LCS, series 2
		Number of positive samples from 24 repetitions					
1	20	24	24	24	24	24	24
2	10	24	23	24	24	24	23
3	5	19	18	19	20	20	17
4	0	-	-	-	-	-	-

Sensitivity depends on the sampling and the final volume of the extracted NA (elution volume).

c. Diagnostic characteristics

Number of samples (n) - 192;

Diagnostic sensitivity (95% CI) - 100% (95.6-100%);

Diagnostic specificity (95% CI) – 100% (96.7-100%).

12. TROUBLESHOOTING

Table 8. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	-	PCR inhibition RNA extraction violation	Repeat RNA extraction Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Telefon: +420 325 209 912

E-mail: ecolidx@ecolidx.com

13. QUALITY CONTROL

Ecoli Dx, s.r.o. declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for In vitro Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our official representative in EU by quality issues of the **eDetect SARS-CoV-2/SARS-CoV Multiplex Kit**:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limitation		Consult instructions for use
Σ	Sufficient for	REF	Catalogue number
	Use by		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
EC REP	Authorized representative in the European Community	CONTROL +	Positive control
NON STERILE	Non-sterile		Do not reuse