DNA-TECHNOLOGY

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CE IVD

For professional use only

AVRI Panel Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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1. INTENDED USE

The **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** is designed to detection of the most common causative agents of acute viral respiratory infections by Real-Time PCR method. Samples are human biological materials: nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm.

Indications for the use:

- ARVI symptoms;
- stay in the centers of infection (for the purpose of early detection of possible infection and prevention of further spread of infection);
- differential diagnosis of ARVI.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **AVRI Panel Multiplex REAL-TIME PCR Detection Kit.**

The **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

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

It is necessary to apply the kit only as directed in this instruction for use.

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 for RNA-containing viruses and DNA for DNA-containing viruses.

The RNA reverse transcription stage and PCR amplification of cDNA/DNA stage are performed in one test tube, that increases the sensitivity of the method, reduces the likelihood of contamination and reduces the time of the study.

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 in the initial heating of the tube.

The **AVRI Panel Multiplex REAL-TIME PCR Detection 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.

The kit includes internal control sample RNA-IC “A” that is intended to assess the quality on nucleic acid (NA) extraction and PCR with reverse transcription. DNA probe used for the detection of the specific amplification product includes the fluorescent dyes Fam, Rox or 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.

Tube No.3 contain additional probe with Rox dye label – “Marker”. It tags the strip orientation.

Upon completion of run, software defines actual position of the strip (by means of “marker” position) relative to the position preset by the operator. If it mismatches, the software suggests rearrangement of the tubes by default. In accordance with the operator, order can be rearranged and saved in new file. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

No. of tube in a strip	Dye label/detection channel				Color labeling of the buffer
	Fam	**Hex**	**Rox**	**Cy5**	
1	Influenza A virus	IC*	SARS-CoV-2 coronavirus, E, N - genes	Influenza B virus	Blue
2	Human parainfluenza virus type 2	IC	Human parainfluenza virus type 4	Human coronavirus 229E	Colorless
3	Human bocavirus	IC	Marker	Human rhinovirus	
4	Human respiratory syncytial virus	IC	–	Human coronavirus HKU1	
5	Human adenovirus	IC	–	Human coronavirus NL63	
6	Human coronavirus OC43	IC	–	Human parainfluenza virus type 3	
7	Human parainfluenza virus type 1	IC	–	–	
8	Human metapneumovirus	IC	–	–	

* - Internal control RNA-IC “A”

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

3. CONTENT

The **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** contains PCR-mix, RT-PCR-buffer, enzyme Taq/RT, internal control RNA-IC ”A”, dilution buffer and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** content, package S (standard) for R3-P439-S3/5EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent or blue liquid under waxy white fraction	2880 µL (15 µL in each tube)	24 8-tube strips
Enzyme Taq/RT	Colorless transparent viscous liquid	100 µL	1 tube
RT-PCR-buffer	Colorless transparent liquid	3.0 mL (1.0 mL in each tube)	3 tubes
Internal control RNA-IC ”A”	Colorless transparent liquid	250 µL	1 tube
Dilution buffer	Colorless transparent liquid	1.0 mL	1 tube
Positive control*	Colorless transparent liquid	320 µL	1 tube
Strip’s caps	24 8-caps		

* - marking as C+ is allowed

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

The **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** is intended for single use and designed for 24 tests (defined samples, positive control and negative control)**.**

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single use sterile containers to collect clinical material;
- Sterile tubes containing transport media: "DNA-Technology" made **STOR-F** (REF P-901-1/1EU) or equivalent for the transportation of the sample.

4.2. NA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II-III;
- Refrigerator;
- High speed centrifuge (RCF(g) 12000-16000) for 1.5 mL tubes;
- Vortex mixer;
- Solid-state thermostat (temperature range 24-65 °C) (for example, TT-2 made by "DNA-Technology");
- Tube rack for 1.5 mL tubes;
- RNase and DNase free 1.5 mL microcentrifuge tubes with caps;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free 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 (volume 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** REF P-002/1EU, **PREP-NA PLUS** REF P-002/2EU, **PREP-NA-S** REF P-007-N/1EU, **PREP-MB-NA-S,** REF P-117-A/9EU, P-118-A/9EU, and **PREP-MB DWP** REF P-119-N/9EU, P-119-P/9EU, P-119-A/9EU DNA/RNA extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile) (if needed);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

When extracting NA from phlegm (method 1):

- 10% trisodium phosphate x 12H2O;
- 1M HCl solution;
- 5.0% chloramines solution;
- Distilled water.

When extracting NA from phlegm (method 2):

- Mucolysin.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Freezing chamber;
- Vortex mixer;
- Vortex rotor for strips;
- 1.5 mL microcentrifuge tubes with caps;
- Tube rack for 1.5 mL tubes;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- 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.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from http://dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of **AVRI Panel Multiplex REAL-TIME PCR Detection Kit**, except the 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, except the Enzyme Taq/RT, 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 kit stored in under undue regime should not be used.

An expired the **AVRI Panel Multiplex REAL-TIME PCR Detection 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 **AVRI Panel Multiplex REAL-TIME PCR Detection 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 **AVRI Panel Multiplex REAL-TIME PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

ATTENTION! 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. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, PCR-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 can not 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 closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. 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 breached;
- 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 **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** is designed to detect NA extracted from the nasopharynx and oropharynx swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm.

Method limitations: for nasopharyngeal and oropharyngeal samples: local application of medications (sprays, drops, creams and ointments) – less than 24 hours before the assay. When using aerosols and other form of medications in the treatment of bronchial asthma, the material for the assay should not be taken earlier than three hours after inhalation or meal.

General requirements

- Use RNase- and DNase-free disposable tips (with filter, except for the supernatant extraction step using an aspirator) during the biomaterial preparation and nucleic acid extraction steps.

- To prevent contamination, always open the cap of the tube you are working with and close it afterwards. It is not allowed to work with several tubes with open caps at the same time.

- If it is necessary to take biomaterial from several biotopes, repeat the procedure, each time taking the material with a new probe into a new tube.

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.

Interfering compounds that can inhibit PCR are:

- endogenous substances (whole blood, leucocytes, mucus);
- exogenous substances (compounds added in the biomaterial during sample preparation (isopropyl alcohol and methyl acetate), locally applied medicines).

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 NA sample, isopropyl alcohol – 100 µL/mL NA sample, methyl acetate – 100 µL/mL NA 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.

Sample collection

Nasopharynx swabs

Take the swab with a dry sterile disposable probe into 1.5 mL plastic tubes with transport medium.

Order of taking:

1. Insert the probe carefully along the outer wall of the nose to a depth of 2-3 cm to the lower shell. Then lower the probe down slightly, insert into the lower nasal passage under the lower nasal conch, after a rotational movement remove along the outer wall of the nose.
2. Open the tube.
3. Put the probe into the tube with transport medium, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the probe from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the probe. Dispose the used probe.
5. Close the tube tightly and mark it.

Oropharynx swabs

Take the swab with a dry sterile disposable probe into 1.5 mL plastic tubes with transport medium.

Order of taking:

1. Take the swab with a probe with a rotational movement from the surface of the tonsils, palatine arches and the back wall of the pharynx.
2. Open the tube.
3. Put the probe into the tube with transport medium, rotate the probe for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the probe from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the probe. Dispose the used probe.
5. Close the tube tightly and mark it.

Bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm

Samples are collected in sterile plastic containers. Transfer 1.0 mL of the sample to a 1.5 plastic test tubes, close the test tube and mark.

Transportation and storage of the samples in accordance with guidance

Type of the sample	Collecting material requirements	Transportation	Storage conditions before transportation	Comments
Nasopharynx and oropharynx swabs	Plastic test tubes and probes for swabs**	4 °C	≤5 days: 4 °C >5 days *: minus 70 °C	Nasopharyngeal and oropharyngeal probes should be placed in the same tube to increase the viral load
Bronchoalveolar lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: minus 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 *: minus 70 °C	
Phlegm	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: minus 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 physiological saline solution (if transportation to the laboratory no more than 24 hours after taking the sample) or a dry probe (if transportation to the laboratory no more than 4 hours after taking the sample).

ATTENTION! Avoid repeated freezing and thawing of samples.

Sample preparation

ATTENTION! For NA extraction, 100 µL of the sample is used. Do not perform centrifugation as a pretreatment of nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate.

You can find the sputum pretreatment procedure in the user manual to the “DNA-Technology” made **PREP-NA, PREP-NA PLUS** extraction kits.

8. PROCEDURE

ATTENTION! The range of causative agents of AVRI including SARS-CoV-2 viral load can vary widely. 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 NA 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 or a special opener (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. NA extraction

It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of NA from the corresponding types of biomaterial.

For NA extraction from the nasopharynx and oropharynx swabs, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm, NA extraction kits are used (see Table 3).

ATTENTION! The volume of obtained NA preparation should be 100 µL. Increasing of the volume of obtained NA preparation will lead to proportional decrease of NA concentration and analysis sensitivity.

Table 3. The reagent kits validated for NA extraction and further study with the **AVRI Panel Multiplex REAL-TIME PCR Detection Kit**

Extraction kit	Biomaterial
PREP-NA and **PREP-NA PLUS**	nasopharynx and oropharynx swabs, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm
PREP-NA and **PREP-NA PLUS** (shortened method in accordance with Annex A)	nasopharynx and oropharynx swabs
PREP-NA-S	nasopharynx and oropharynx swabs
PREP-MB-NA-S	nasopharynx and oropharynx swabs
PREP-MB DWP	nasopharynx and oropharynx swabs

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

ATTENTION! The resulting NA preparation must be used immediately for RT-PCR. If it is needed, the resulting NA 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

ATTENTION! Do not perform centrifugation as a pretreatment of nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate.

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

NOTE. The volume of the resulting NA preparation should not exceed 100 µL, with a smaller amount, it is necessary to bring the volume to the required level before performing RT-PCR, using a dilution buffer from the **AVRI Panel Multiplex REAL-TIME PCR Detection Kit**.

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

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 **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** should be used as an internal control sample.

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

ATTENTION! The internal control (RNA-IC) and internal control (DNA-IC) from the **PREP-NA DNA/RNA Extraction Kit** are not used.

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.

ATTENTION! Independently of DNA/RNA extraction kit used, it is obligatorily to use a negative control sample that must go through all stages of extraction simultaneously with nucleic acid extraction from biological 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 included in the corresponding extraction kit.

8.4. PCR with Reverse Transcription (RT-PCR)

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

ATTENTION! Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.4.1 Mark the required number of strips with paraffin sealed PCR-mix: 1 strip for each sample to be tested, 1 strip for positive control (C+) and 1 strip for negative control (C-).

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

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

ATTENTION! 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 x (N+1) µL of RT-PCR-buffer;
- 0.5 x (N+1) µL of Enzyme Taq/RT,
- N is a quantity of tubes in the marked strips.

Example: to test 6 samples, mark 8 strips. Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT for 65 (64+1) tubes. Mix 975 µL of RT-PCR-buffer and 32.5 µL of Enzyme Taq/RT.

ATTENTION! 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 for 3-5 seconds, then spin 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. Close the strips.

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

ATTENTION! If magnetic particle-based reagent kits (**PREP-MB-NA-S, PREP-MB DWP**) are used for the NA extraction, the tubes with the NA preparation must be placed in a magnetic rack after shaking. If the supernatant containing the isolated NA is transferred to new tubes after extraction, centrifugation is performed for 3-5 seconds on a vortex mixer.

ATTENTION! Open the tube, add NA sample (or control sample), then close the strip before proceeding to the next strip to prevent contamination. Close the strips tightly. Use filter tips

8.4.7 Add 10 µL of the NA sample into corresponding tubes. Do not add NA into the “C-”, “C+” strips. Avoid paraffin layer break.

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

8.4.9 Spin down the strips for 3-5 seconds to collect drops.

8.4.10 Set the strips into the Real-time Thermal Cycler.

8.4.11 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the strips in the thermal unit (see 8.4.10) and run PCR. See Table 4.

Table 4. The program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	35	20	0	1		Cycle
2	95	5	0	1		Cycle
3	94	0	10	5		Cycle
	64	0	10		v	
4	94	0	5	45		Cycle
	64	0	10		v	
5	80	0	1	1		Cycle
6	10[1]	…	…	Holding		Holding

√ - optical measurement
[1] – holding at 25°C is allowed

9. CONTROLS

The **AVRI Panel Multiplex REAL-TIME PCR Detection 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 NA extraction and RT-PCR To reveal possible contamination a negative control is required.

ATTENTION! A negative control sample should go through all stages of NA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control sample in volumes as indicated in supplied instructions.

For **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** 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 absent and for internal control is present.

For **AVRI Panel Multiplex REAL-TIME PCR Detection Kit** the test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

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.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

Registration of the RT-PCR results is held in automatic mode. Analysis will be performed by Real-Time PCR application.

1 The interpretation of assay results for control samples should be performed in accordance with Table 5.

Table 5. The interpretation of assay results for control samples

Detection channel				**Interpretation**
Fam	Hex	Rox	Cy5	
Positive control sample				
Cp is specified (Strip test tubes No.1-8)	Cp is not considered	**Cp is specified (Strip test tubes No.1-2)**	**Cp is specified (Strip test tubes No.1-6)**	Positive result The results are valid
Negative control sample				
Cp is not specified	**Cp is specified**	Cp is not specified	Cp is not specified	Negative result The results are valid

2 In biological samples containing the nucleic acids of one or more detectable viruses with the reagent kit, positive amplification results ("+") of specific product genomic fragments must be recorded for the corresponding detection channels (Fam, Rox or Cy5).

3 The results are interpreted according to Table 6. It is necessary to take into account the possibility of the presence of several viruses causing ARVI in the sample, including those detected in the same amplification test tube.

4 Biological samples containing no nucleic acids of viruses detected by the reagent kit and a negative control sample should have positive amplification results for RNA-IC ”A” (Hex detection channel) and negative amplification results ("-") for genomic fragments of specific products (Fam, Rox, and Cy5 channels).

The result is rated as unreliable by the software if there are no positive results in the amplification tube for all channels.

5 Unreliable results may be due to the presence of inhibitors in the nucleic acid preparation obtained from clinical material; errors in the preanalytical step, incorrect execution of the assay protocol, non-compliance with the amplification temperature regime, etc. In this case, either the nucleic acid preparation is extracted again or clinical material is taken again (performed sequentially).

6 If there is no positive result (Fam, Rox and Cy5 channels) in the positive control sample, the results of the whole run are rejected.

7 If a positive result (on Fam, Rox or Cy5 channels) is found in a negative control sample (K-), the results of the whole run are rejected. In this case, special measures must be taken to detect and eliminate possible contamination.

Table 6. The interpretation of assay results for PCR

Detection channel				**Interpretation**
Fam	Hex	Rox	Cy5	
Strip test tube No.1				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Influenza A virus RNA is detected**
Cp is not specified	Is not considered	**Cp is specified**	Cp is not specified	**SARS-CoV-2 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Influenza B virus RNA is detected**
Strip test tube No.2				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human parainfluenza virus type 2 RNA is detected**
Cp is not specified	Is not considered	**Cp is specified**	Cp is not specified	**Human parainfluenza virus type 4 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus 229E RNA is detected**
Strip test tube No.3				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human bocavirus DNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human rhinovirus RNA is detected**
Strip test tube No.4				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human respiratory syncytial virus RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus HKU1 RNA is detected**
Strip test tube No.5				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human adenovirus DNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus NL63 RNA is detected**
Strip test tube No.6				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human coronavirus OC43 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human parainfluenza virus type 3 RNA is detected**
Strip test tube No.7				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human parainfluenza virus type 1 RNA is detected**
Strip test tube No.8				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human metapneumovirus RNA is detected**
For all test tubes				
Cp is not specified	**Cp is specified**	Cp is not specified	Cp is not specified	Target viruses RNA is not detected
Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	Unreliable result

ATTENTION! A single negative test result, especially if it is a sample from the upper respiratory tract, does not exclude infection.

ATTENTION! Negative results should not be used as the sole basis for making a decision about the treatment of patients.

If in the samples of human biological material the Real-time PCR thermal cycler registers an increase in fluorescence for the specific product earlier than 25 cycle for Cp, this indicates a high initial NA concentration of the corresponding pathogen.

In this case, it is possible to obtain a false negative result during mixed infection for a pathogen whose NA is present in a low concentration. To exclude false negative results, it is recommended to repeat RT-PCR for the extracted NA preparation using the kit for individual detection of the corresponding virus.

11. SPECIFICATIONS

a. The analytical specificity of the **AVRI Panel Multiplex REAL-TIME PCR Detection 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.

In the samples of human biological material with target viruses NA, the thermal cycler should register an increase in fluorescence on the corresponding detection channels.

In the samples of human biological material free of target viruses NA, the thermal cycler should register an increase in fluorescence on the Hex detection channel, the increase in fluorescence on the Fam, Rox, and Cy5 channels should be absent.

The absence of nonspecific positive amplification results was shown in the presence of nucleic acids of typical representatives of *Streptococcus* spp., *Staphylococcus* spp., *Klebsiella* spp., *Bordetell*a spp., *Candida* spp., *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, *Morganella morganii*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Proteus mirabilis*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*., *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes, Streptococcus agalactiae*, Enterovirus, HSV 1, 2 and human DNA at more than 750 ng per amplification tube.

Specific amplification results were detected in the presence of detectable analytes in the NA sample: SARS-CoV-2 (examined under the international system QCMD (Quality Control for Molecular Diagnostics), UK), Influenza B virus, Influenza A virus of different subtypes (including (H1N1)pdm09, H3N2), respiratory syncytial virus, parainfluenza viruses types 1-4, rhinovirus, adenovirus, metapneumovirus, HKU1, NL63, OC43, 229E coronaviruses, bocavirus.

The absence of cross-reactivity of each of the systems included in the reagent kit with respect to pathogens determined by other systems was shown.

b. Analytical sensitivity is 10 copies of NA per amplification tube for Influenza A virus, Influenza B virus, SARS-CoV-2 coronavirus and 20 copies of NA per amplification tube for other viruses. Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

The analytical sensitivity of 10 or 20 NA copies per amplification tube corresponds to the following values of NA concentration in the sample when using the nucleic acid extraction kits made by DNA-Technology with the final volume of NA preparation equal to 100 µL:

Analytes	Analytical sensitivity			
	Influenza A virus, Influenza B virus, SARS-CoV-2		Human parainfluenza virus type 1-4, Human coronavirus 229E, OC-43, HKU-1, NL-63, Human respiratory syncytial virus, Human metapneumovirus, Human rhinovirus, Human adenovirus, Human bocavirus	
	10 copies per amplification tube		20 copies per amplification tube	
	Volume of the obtained NA preparation - 100 µL			
Biomaterial/NA extraction kit	**PREP-NA, PREP-NA PLUS**	**PREP-NA-S, PREP-MB-NA-S, PREP-MB DWP**	**PREP-NA, PREP-NA PLUS**	**PREP-NA-S, PREP-MB-NA-S, PREP-MB DWP**
Swabs from nasopharynx, oropharynx in 500 µL of transport medium	$2x10^3$ copies/mL sample	$2x10^3$ copies/mL sample	$4x10^3$ copies/mL sample	$4x10^3$ copies/mL sample
Bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate	$2x10^3$ copies/mL sample	Not used	$4x10^3$ copies/mL sample	Not used
Phlegm (pretreated with Na_3PO_4,. method 1)	$4x10^3$ copies/mL sample	Not used	$8x10^3$ copies/mL sample	Not used
Phlegm (pretreated with mucolysin, method 2)	$1x10^4$ copies/mL sample	Not used	$2x10^4$ copies/mL sample	Not used

c. Diagnostic characteristics

Biomaterial	**Number of samples (n)**	**Diagnostic sensitivity (%)**	**Diagnostic specificity (%)**
Swabs from nasopharynx, oropharynx	82	100% (95.32 – 100)	100% (99.70– 100)
Bronchoalveolar lavage	29	100% (87.23 – 100)	100% (99.16 – 100)
Endotracheal aspirate	18	100% (79.41 – 100)	100% (98.65 – 100)
Nasopharyngeal aspirate	21	100% (85.18 – 100)	100% (98.83– 100)
Phlegm	76	100% (95.26 – 100)	100% (99.68 – 100)
Sum	226	100% (98.31 – 100)	100% (99.89– 100)

d. Within-batch and between-batch precision

Within-batch precision (95% CI) – 100 % (92.45-100%).

Between-batch precision (95% CI) - 100 % (92.45-100%).

12. TROUBLESHOOTING

Table 7. Troubleshooting

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

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

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC 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 **AVRI Panel Multiplex REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Manufacturer
	Temperature limit		Date of manufacture
	Contains sufficient for <n> tests		Consult instructions for use
	Use-by date	REF	Catalogue number
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Caution

Annex A

Shortened method for the NA extraction from the tested material (nasopharynx and oropharynx swabs) using PREP-NA and PREP-NA PLUS DNA/RNA Extraction Kit

ATTENTION! The lysis buffer can form the precipitate. Dissolve it at 65 °C for 10 minutes prior to use.

- Mark the required number of 1.5 mL tubes according to the number of samples to be analyzed and negative control (C-).
- Vortex the tube with RNA-IC "A" (from **AVRI Panel Multiplex REAL-TIME PCR Detection Kit**), spin the tube for 3-5 seconds. Add 10 µL of the RNA-IC "A" into the each tube.
- Add 300 µL of the lysis buffer into the each tube avoiding contact of the pipette tip with an edge of the tube.
- Add 100 µL of the sample into corresponding tubes. Add 100 µL negative control into the tube marked "C-".
- Close the tubes tightly. Vortex the tubes for 3-5 seconds.
- Incubate the tubes for 5 minutes at 65 °C.
- Spin the tubes for 3-5 seconds.
- Add 400 µL of the precipitation buffer. Close the tubes tightly and vortex for 3–5 seconds.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 10 minutes at room temperature (18-25 °C).
- Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.
- Add 500 µL of the wash solution №1 to the precipitate, close tubes and mix by inverting the tube 3-5 times.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 1 minute at room temperature (18-25 °C).
- Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.
- Add 300 µL of the wash solution №2 to the precipitate, close tubes and mix by inverting the tube 3-5 times.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 1 minute at room temperature (18-25 °C).
- Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample. It is allowed to leave the precipitate covering with liquid in the volume of no more than 20-30 µL.
- Open the tubes and dry the precipitate at 65 °C for 5 minutes.
- Add 50 µL (**PREP-NA**) or 100 µL (**PREP-NA PLUS**) of the dilution buffer to the precipitate. Vortex the tubes for 3-5 seconds and spin down the drops by centrifuging for 3-5 seconds.

NOTE. The volume of the resulting NA preparation should not exceed 100 µL, with a smaller amount, it is necessary to bring the volume to the required level before performing RT-PCR, using a dilution buffer from the **AVRI Panel Multiplex REAL-TIME PCR Detection Kit**.

- Incubate the tubes for 5 minutes at 65 °C. Vortex the tubes for 3-5 seconds.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 30 seconds at room temperature (18-25 °C).

The NA preparation is ready for RT-PCR.

ATTENTION! The resulting NA preparation must be used immediately for RT-PCR. If it is needed, the resulting NA 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.