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**For research use only**

## **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit**

### **INSTRUCTION FOR USE**



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R3-P449-S3/9EU  
R3-P449-23/9EU



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## 1. INTENDED USE

The **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** is designed to detect the RNA of Influenza A virus and Influenza B virus in human biological material (nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm) by Reverse Transcription (RT) and Polymerase Chain Reaction (PCR) methods.

Indications for the use:

- AVRI symptoms or contacts with AVRI patients;
- 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 AVRI.

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

The **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** can be used in 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.

The principle of the method is based on the use of the process of reverse transcription of RNA and subsequent amplification of cDNA, which consists of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent extension of polynucleotide chains from these primers with Taq polymerase.

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

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 or Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only when paraffin melts or after the Taq-polymerase or antibody complex temperature dissociating, which excludes the possibility of non-specific annealing of primers on the DNA target during the initial warm-up of the test tube.

The **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** includes the Internal control (RNA-IC "A"), which is intended to assess the quality of the RNA extraction, reverse transcription and polymerase chain reaction.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the reaction mixture for amplification. When a specific product is formed, the DNA probe is destroyed and the quencher stops affecting the fluorescent label, which leads to an increase in the fluorescence level. The number of destroyed probes (and hence the fluorescence level) increases in proportion to the number of specific amplicons formed. The fluorescence level is measured at each amplification cycle in real time.

DNA probe used for the detection of the Influenza A virus cDNA products is labeled with fluorescent reporter dye Fam.

DNA probe used for the detection of the Influenza B virus cDNA products is labeled with fluorescent reporter dye Cy5.

DNA probe used for the detection of the internal control (RNA-IC “A”) amplification product is labeled with the fluorescent reporter dye Hex. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

| Fam/Green         | Hex/Yellow | Rox/Orange | Cy5/Red           | Cy5.5/Crimson |
|-------------------|------------|------------|-------------------|---------------|
| Influenza A virus | IC*        | -          | Influenza B virus | -             |

\* - Internal control RNA-IC “A”

The automatic analysis for **Influenza A/B virus Multiplex REAL-TIME-PCR Detection Kit** is available on “DNA-Technology” made DTprime<sup>1</sup> and DTlite<sup>2</sup> REAL-TIME Thermal Cyclers; the latest version of the software is available for download at <https://www.dna-technology.com/software>.

The **Influenza A/B virus Multiplex REAL-TIME-PCR Detection Kit** is also approved for use with Rotor-Gene Q (QIAGEN) real-time thermal cyclers.

### 3. CONTENT

The detailed description of content is represented in Tables 2-3.

Table 2. The **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** content, package S, strips for R3-P449-S3/9EU

| Reagent                     | Description   | Nominal volume                  | Amount                |
|-----------------------------|---|---------------------------------|-----------------------|
| Paraffin sealed PCR-mix     | Colorless or slightly pink transparent liquid under waxy white fraction | 1440 µL<br>(15 µL in each tube) | tubes, 12 strips of 8 |
| RT-PCR-buffer               | Colorless transparent liquid  | 1.62 mL                         | 1 tube                |
| Enzyme Taq/RT               | Colorless transparent viscous liquid                                    | 55 µL                           | 1 tube                |
| Internal control RNA-IC “A” | Colorless transparent liquid  | 1.0 mL                          | 1 tube                |
| Positive control*           | Colorless transparent liquid  | 130 µL                          | 1 tube                |
| Strip caps                  | 12 strips of 8  |                                 |                       |

\* - marking as C+ is allowed

<sup>1</sup> - supported by 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.

<sup>2</sup> - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.

Table 3. The **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** content, package S, tubes for R3-P449-23/9EU

| Reagent                     | Description   | Nominal volume                  | Amount              |
|-----------------------------|---|---------------------------------|---------------------|
| Paraffin sealed PCR-mix     | Colorless or slightly pink transparent liquid under waxy white fraction | 1440 µL<br>(15 µL in each tube) | 96 individual tubes |
| RT-PCR-buffer               | Colorless transparent liquid  | 1.62 mL                         | 1 tube              |
| Enzyme Taq/RT               | Colorless transparent viscous liquid                                    | 55 µL                           | 1 tube              |
| Internal control RNA-IC "A" | Colorless transparent liquid  | 1.0 mL                          | 1 tube              |
| Positive control*           | Colorless transparent liquid  | 130 µL                          | 1 tube              |

\* - marking as C+ is allowed

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

The kit is intended for single use and designed for 96 tests (including one positive control and one negative control in each run). It is recommended to perform no more than 12 runs.

#### 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 medium: "DNA-Technology" made **STOR-F** ( [REF](#) P-901-1/1EU) or physiological saline solution (Sterile) or equivalent for the transportation of the sample.

##### 4.2. RNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 40-95 °C);
- Tube rack for 1.5 mL tubes;
- DNase and RNase free 1.5 mL microcentrifuge tubes with caps;
- 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-S** [REF](#) P-007-N/1EU, **PREP-MB DWP** [REF](#) P-119-A/9EU, [REF](#) P-119-N/9EU, [REF](#) P-119-P/9EU, **PREP-MB-NA-S** [REF](#) P-117-A/9EU, [REF](#) P-118-A/9EU DNA/RNA extraction kits are recommended);

- Physiological saline solution 0.9% NaCl (Sterile);
- 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 12H<sub>2</sub>O;
- 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 (in case of using package S, strips REF R3-P449-S3/9EU);
- PCR tube rack for 0.2 mL tubes or strips;
- 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);
- Pipette stand;
- Tube rack for 1.5 mL tubes;
- 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 <https://www.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 **Influenza A/B virus 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 Enzyme Taq/RT must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The excessive temperature and light can be detrimental to product performance.

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 thermobox 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 thermobox 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.

The kit transported under undue regime should not be used.

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 **Influenza A/B virus 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 **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

## 6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

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 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 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 **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** is designed to detect RNA extracted from nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm.

**Method limitations:** 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

1. 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.
2. When adding solution to a tube containing biological material, carefully introduce liquids without touching the walls of the tubes. If you touch the wall of the tube, change the tip. The tip should be changed each time the solution is removed from the sample.
3. To prevent contamination, always open only 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.

### Interfering Substances

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

PCR-inhibiting interfering substances include:

- endogenous substances (whole blood, leukocytes, mucus);
- exogenous (substances added to biomaterial samples during sample preparation (isopropyl alcohol and methyl acetate), topical medications).

The maximum concentrations of interfering agents at which no effect on amplification was observed were: hemoglobin, 0.35 mg/mL of RNA sample; isopropyl alcohol, 100 µL/mL of RNA sample; methyl acetate, 100 µL/mL of RNA sample.

Interfering effects of substances such as leukocytes, mucus, and topical medications, which may be present in clinically relevant concentrations in biomaterial samples, were not observed.

To reduce the amount of interfering substances that inhibit PCR, it is necessary to follow the rules of biological material collection. If the presence of large amounts of PCR inhibitors in a sample is suspected, it is advisable to choose nucleic acid extraction methods that allow maximum removal from the sample; express methods of nucleic acid extraction are not recommended.

To avoid RT-PCR inhibition, it is recommended to use “DNA-Technology” made **STOR-F** ( **REF** P-901-1/1EU) or physiological saline solution (sterile) with **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit**.

## **Sample collection**

### **Phlegm**

Material is taken into single-use graduated sterile vials with wide neck and screw caps with a volume of not less than 50 mL in an amount not less than 1.0 mL.

After the sample collection the vial is tightly closed and labeled.

#### **NOTE:**

Phlegm must be pretreated. The procedure for phlegm pretreatment with a solution of trisubstituted sodium phosphate (Na<sub>3</sub>PO<sub>4</sub>) or mucolysin is given in the instructions to the reagent kit (**PREP-NA DNA/RNA Extraction Kit**, "DNA-Technology R&P", LLC).

### **Bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate**

Material is taken into single-use graduated sterile vials with wide neck and screw caps with a volume of up to 50 mL. After the sample collection the vial is tightly closed and labeled.

### **Nasopharyngeal and oropharyngeal swabs**

Material collection is performed using specific medical devices depending on the source of the biological material according to the established procedure.

After collecting the material, transfer the probe to a tube of transport medium intended by the manufacturer for transporting and storing biological material samples for PCR assays, and rinse it thoroughly in liquid for 10-15 seconds, avoiding splashing the liquid.

Remove the probe from the solution and, rotating it against the inner wall of the tube above the level of the solution, squeeze out the excess liquid. Completely remove the probe from the tube and discard.

Close the lid of the tube tightly and label the tube.

If biomaterial from more than one biotope needs to be collected, repeat the procedure, each time taking material with a new probe into a new tube.

## **Transportation and storage of the samples in accordance with guidance**

### **Phlegm**

Phlegm samples are allowed to be stored:

at room temperature (18 °C – 25 °C) for up to 6 hours;

at 2 °C – 8 °C for up to 3 days.

### **Bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate**

Bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate are allowed to be stored:

at 2 °C – 8 °C for up to 24 hours;

at minus 18 °C to minus 20 °C or up to 1 week.

**ATTENTION!** Avoid repeated freezing and thawing of samples.

### **Nasopharyngeal and oropharyngeal swabs**

The transportation and storage conditions of nasopharyngeal and oropharyngeal swabs are determined by the instructions for the recommended transport media and kits for RNA isolation.

Further processing of the above types of biological material is performed according to the instructions for the used kits for RNA isolation.

## 8. PROCEDURE

**ATTENTION!** The range of influenza viral load can vary widely. In clinical laboratory practice the risk of cross-contamination between samples remains a serious danger, especially during aliquoting and RNA extracting. Cross-contamination with high-copy nucleic acids can lead to sporadic false-positive results.

To prevent cross-contamination 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 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).

### Assay procedure

#### 8.1 RNA extraction

For RNA extraction from the nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm RNA extraction kits are used (see Table 4).

Table 4. The reagent kits validated for RNA extraction and further study with the **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit**.

| Reagent kit   | Biomaterial   |
|---|---|
| <b>PREP-NA DNA/RNA Extraction Kit</b>                           | nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm |
| <b>PREP-NA</b><br>(shortened method in accordance with Annex A) | nasopharyngeal and oropharyngeal swabs  |
| <b>PREP-NA-S</b>  | nasopharyngeal and oropharyngeal swabs  |
| <b>PREP-MB-NA-S</b>   | nasopharyngeal and oropharyngeal swabs  |
| <b>PREP-MB DWP</b>  | nasopharyngeal and oropharyngeal swabs  |

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

### ATTENTION!

The volume of the resulting RNA preparation should not exceed 50 µL. If the **PREP-MB-NA-S** and **PREP-MB DWP** kits are used for RNA isolation, the volume can be increased up to 100 µL.

The resulting RNA preparation must be used in the course of 2 hours 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.

#### 8.1.1 The features of biomaterial preparation for Influenza A/B virus RNA testing

**ATTENTION!** 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.

## 8.1.2 The use of control samples at the stage of nucleic acid extraction

### 8.1.2.1 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 **Influenza A/B virus 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.

**NOTE** – Internal controls (RNA-IC and DNA-IC) from the **PREP-NA** extraction kits are not used during the RNA isolation.

### 8.1.2.2 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.

In the nucleic acid isolation step, be sure to prepare a negative control and run it through all isolation steps simultaneously with RNA isolation 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.

## 8.2 PCR with Reverse Transcription (RT-PCR)

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

**ATTENTION!** When using package S (R3-P449-S3/9EU), strips, strictly observe the completeness of the strips and caps to them. Do not use the caps to the strips of the other kits!

8.2.1 Mark the required number of the tubes/ stripped 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.

8.2.2 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.

8.2.3 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 the samples to be tested taking into 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.

**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.

8.2.4 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.

**ATTENTION!** Mixture can be stored at 2 °C – 8 °C for up to 1 hour.

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

8.2.6 Vortex the tubes with “C-” and “C+” for 3-5 seconds and spin down drops for 1-3 seconds.

**ATTENTION!** Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

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

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

8.2.9 Add 10 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.2.10 Spin down the strips for 3-5 seconds to collect drops (when using the Rotor-Gene Q for PCR, centrifugation is not necessary).

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

8.2.12 Launch the operating software for DT instrument<sup>3</sup>. Add corresponding test<sup>4</sup>, 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 8.2.11) and run PCR. See Table 5.

For use with Rotor-Gene Q thermal cycler consult user manual for devices. See Table 6.

Table 5. The PCR program for DTprime and DTlite 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   |                  | √                   |                  |
| 4                       | 94              | 0    | 5    | 45               |                     | Cycle            |
|                         | 64              | 0    | 10   |                  | √                   |                  |
| 5                       | 80              | 0    | 1    | 1                |                     | Cycle            |
| 6                       | 10              | ...  | ...  | Holding          |                     | Holding          |
| √ - optical measurement |                 |      |      |                  |                     |                  |

Table 6. Amplification program for Rotor-Gene Q thermal cycler

| Cycling  | Temperature | Hold time | Cycle repeats |
|--|-------------|-----------|---------------|
| Cycling  | 32 deg      | 1200 sec  | 1 time        |
| Cycling 2  | 95 deg      | 300 sec   | 1 time        |
| Cycling 3  | 94 deg      | 10 sec    | 50 times      |
|  | 60 deg √    | 15 sec    |               |
| √ - optical measurement, set the fluorescence measurement (Acquiring) on the channels Green (Fam), Yellow (Hex), Orange (Rox) and Red (Cy5) at 60 °C |             |           |               |

<sup>3</sup> Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

<sup>4</sup> Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website <https://www.dna-technology.com/assaylibrary>.

For CITO diagnostics, it is allowed to use a shortened amplification program for the “DTprime” detection thermal cycler (Annex A, Table A.1).

**ATTENTION!** It is not allowed to use a shortened amplification program in routine assays.

## 9. CONTROLS

The **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the virus genome. It is produced by 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 RNA extraction, reverse transcription and polymerase chain reaction. To reveal possible contamination a negative control is required.

**ATTENTION!** A negative control sample should go through all stages of RNA extraction. Physiological saline solution can be used as a negative control sample.

For **Influenza A/B virus 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 **Influenza A/B virus 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 observed.

If positive control (C+) does not have positive result, it is necessary to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling requirements.

If negative control (C-) has positive result, all results of current PCR run are considered false. The retesting and decontamination are required.

## 10. DATA ANALYSIS

Registration of the PCR results is held in automatic mode. Interpretation of the PCR results should be performed accordance to the Table 7.

Table 7. Interpretation of PCR results

| Detection channel       |                        |                        | Result interpretation  |
|-------------------------|------------------------|------------------------|--|
| Fam/Green               | Hex/Yellow             | Cy5/Red                |  |
| Samples                 |                        |                        |  |
| Cp/Ct is specified      | Is not considered      | Cp/Ct is not specified | Influenza A virus RNA is detected                                      |
| Cp/Ct is not specified  | Is not considered      | Cp/Ct is specified     | Influenza B virus RNA is detected                                      |
| Cp/Ct is specified      | Is not considered      | Cp/Ct is specified     | Influenza A virus RNA is detected<br>Influenza B virus RNA is detected |
| Cp/Ct is not specified  | Cp/Ct is specified     | Cp/Ct is not specified | RNA of Influenza A and B viruses is not detected                       |
| Cp/Ct is not specified  | Cp/Ct is not specified | Cp/Ct is not specified | Invalid result   |
| Negative control sample |                        |                        |  |
| Cp/Ct is not specified  | Cp/Ct is specified     | Cp/Ct is not specified | Negative result.<br>The results of PCR run are valid.                  |
| Positive control sample |                        |                        |  |
| Cp/Ct is specified      | Cp/Ct is not specified | Cp/Ct is specified     | Positive result.<br>The results of PCR run are valid.                  |

**10.1** The results of the run are valid if the conditions for control result interpretation are fulfilled.

**10.2** In biological samples containing RNA of viruses detected by the kit, the detection thermal cycler registers an exponential increase in the fluorescence level in the corresponding detection channel ((Fam/Green or Cy5/Red))

**10.3** In biological samples containing no RNA of viruses detected by the reagent kit and in the negative control sample, the detection thermal cycler registers an exponential increase in the fluorescence level in the Hex/Yellow channel (internal control sample), there is no exponential increase in fluorescence in the Fam/Green and Cy5/Red channels.

**10.4** The result is considered by the software to be invalid if there is no exponential increase in the fluorescence level for the specific product (Fam/Green and Cy5/Red channels) and for the internal control sample (Hex/Yellow channel).

**10.5** Unreliable result can be related to the presence of PCR inhibitors in RNA sample, incorrect analysis procedure, violation of the amplification temperature regimen, etc.

In case of suspicion for the presence of inhibitors in sample, it is necessary to repeat nucleic acid extraction from sample, or repeat sampling (performed sequentially).

## Warnings

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

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

If an increase in fluorescence for a specific product is detected before cycle 25 of Cp (Cp less than 25) or before cycle 22 of Ct (Ct less than 22) in a biological sample, this indicates a high initial RNA concentration of the corresponding pathogen. In this case, it is possible to obtain a false-negative result for a pathogen which RNA is present at a low concentration in the mixture. To rule out false negatives, it is recommended to repeat the RT-PCR for the isolated RNA preparation using a kit for the individual detection of the corresponding virus.

## 11. SPECIFICATIONS

a. The analytical specificity of the **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit** was assessed by bioinformatic 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.

The absence of cross-reactivity of each of the oligonucleotide systems included in the kit with respect to viruses determined by the other systems has been shown.

There are not non-specific positive results of amplification of RNA sample in the presence Human Coronavirus 229E, Human Coronavirus HKU-1, Human Coronavirus NL-63, Human Coronavirus OC-43, Human Metapneumovirus, Human Parainfluenza virus type 1, Human Parainfluenza virus type 2, Human Parainfluenza virus type 3, Human Parainfluenza virus type 4, Human Rhinovirus, MERS-CoV, Respiratory syncytial virus, SARS-CoV-2, DNA Human Adenovirus, Human Bocavirus, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydophila pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Staphylococcus aureus* (methicillin-resistant), *Streptococcus pneumoniae*, as well as human DNA in concentrations up to  $1.0 \times 10^8$  copies/mL of the sample.

b. Analytical sensitivity. LOD (limit of detection) is 10 NA copies per amplification tube.

The analytical sensitivity was determined by analyzing serial dilutions of two series of a laboratory control (LC).

The RNA analytical sensitivity in a biomaterial sample depends on the sample preparation method and the final volume of isolated RNA (elution volume).

The detection limit of 10 nucleic acid copies per amplification tube corresponds to the following values of RNA concentration in the sample when nucleic acid extraction kits are used:

| Biomaterial   | PREP-NA<br>(obtained preparation<br>volume 50 µL) | PREP-NA-S<br>(obtained preparation<br>volume 50 µL) | PREP-MB-NA-S;<br>PREP-MB DWP<br>(obtained preparation<br>volume 100 µL) |
|---|---|---|---|
| Naso-/oropharyngeal swabs in<br>500 µL of transport medium;         | 1000 copies/mL of<br>sample                       | 1000 copies/mL of<br>sample                         | 2000 copies/mL of sample  |
| Bronchoalveolar lavage,<br>endotracheal, nasopharyngeal<br>aspirate | 1000 copies/mL of<br>sample                       | Not applicable                                      | Not applicable  |
| Phlegm<br>(pretreated with Na <sub>3</sub> PO <sub>4</sub> )        | 2000 copies/mL of<br>sample                       | Not applicable                                      | Not applicable  |
| Phlegm<br>(pretreated with mucolysin)                               | 5000 copies/mL of<br>sample                       | Not applicable                                      | Not applicable  |

**c. Diagnostic characteristics**

| Type of biomaterial   | Virus                    | Diagnostic sensitivity            | Diagnostic specificity            |
|---|--------------------------|-----------------------------------|-----------------------------------|
| Naso-/oropharyngeal swabs                                     | Influenza A virus        | 100%<br>(90.51-100)               | 100%<br>(93.84-100)               |
|   | Influenza B virus        | 100%<br>(89.42-100)               | 100%<br>(94.22-100)               |
| Bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate | Influenza A virus        | 100%<br>(88.43-100)               | 100%<br>(92.89-100)               |
|   | Influenza B virus        | 100%<br>(88.43-100)               | 100%<br>(92.89-100)               |
| Phlegm  | Influenza A virus        | 100%<br>(90.26-100)               | 100%<br>(89.72-100)               |
|   | Influenza B virus        | 100%<br>(89.72-100)               | 100%<br>(90.26-100)               |
| <b>All biomaterial</b>  | <b>Influenza A virus</b> | <b>100%</b><br><b>(96.48-100)</b> | <b>100%</b><br><b>(97.44-100)</b> |
|   | <b>Influenza B virus</b> | <b>100%</b><br><b>(96.27-100)</b> | <b>100%</b><br><b>(97.54-100)</b> |

## 12. TROUBLESHOOTING

Table 8. Troubleshooting

|    | Result  | Possible cause  | Solution  |
|----|---------|---|---|
| C+ | -       | Operation error<br>PCR inhibition<br>Violation of storage and handling requirements | Repeat whole test<br><br>Dispose current batch              |
| C- | +       | Contamination   | Dispose current batch<br>Perform decontamination procedures |
| IC | Invalid | PCR inhibition  | Repeat whole test<br>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](mailto:hotline@dna-technology.ru)

<https://www.dna-technology.com>

### 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 IVD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit**.

Technical support:

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<http://www.dna-technology.com>

#### 14. KEY TO SYMBOLS

|   |                                   |  |                              |
|---|-----------------------------------|--|------------------------------|
|  | For research use only             |  | Manufacturer                 |
|  | Temperature limit                 |  | Date of manufacture          |
|  | Contains sufficient for <n> tests |  | Consult instructions for use |
|  | Use-by date                       |  | Catalogue number             |
|  | Batch code                        |  | Keep away from sunlight      |
|  | Version                           |  | Caution                      |
|  | Do not reuse                      |  |                              |

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R3-P449-S3/9EU  
R3-P449-23/9EU

**VER**

847.2022.12.27

### Shortened method for the DNA/RNA extraction from nasopharyngeal and oropharyngeal swabs using PREP-NA 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 **Influenza A/B virus Multiplex REAL-TIME PCR Detection Kit**). Add 10 µL of the RNA-IC "A" into each tube.
- Add 300 µL of the lysis buffer into each tube avoiding contact of the pipette tip with an edge of the tube.
- Add 100 µL of sample into marked tubes. Add 100 µL of 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.
- Vortex the tubes for 3-5 seconds.
- Add 400 µL of the precipitation buffer. Close the tubes tightly and vortex them 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 a 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 a 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 a 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 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.
- 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.

The obtained RNA preparation should be used for reverse transcription and polymerase chain reaction within two hours. To be able to repeat the study, the remaining RNA should be immediately placed in a freezer and stored at no more than minus 18 °C for no more than 7 days without defrosting before staging.

Table A.1 – Shortened amplification program for the “DTprime” detection thermal cycler (only allowed for CITO diagnostics)

| Step                   | Temperature, °C | Min. | Sec. | Number of cycles | Optical measurement | Type of the step |
|------------------------|-----------------|------|------|------------------|---------------------|------------------|
| 1                      | 35              | 15   | 0    | 1                |                     | Cycle            |
|                        |                 |      |      |                  |                     |                  |
| 2                      | 92              | 0    | 30   | 1                |                     | Cycle            |
|                        |                 |      |      |                  |                     |                  |
| 3                      | 92              | 0    | 10   | 8                |                     | Cycle            |
|                        | 64              | 0    | 15   |                  | √                   |                  |
|                        |                 |      |      |                  |                     |                  |
| 4                      | 90              | 0    | 5    | 40               |                     | Cycle            |
|                        | 64              | 0    | 15   |                  | √                   |                  |
|                        |                 |      |      |                  |                     |                  |
| 5                      | 64              | 0    | 5    | 1                |                     | Cycle            |
|                        |                 |      |      |                  |                     |                  |
| 6                      | 10              | ...  | ...  | Holding          |                     | Holding          |
| √- optical measurement |                 |      |      |                  |                     |                  |