DNA-TECHNOLOGY

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CE IVD

For professional use only

Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit

USER MANUAL

EC REP

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1. INTENDED USE

The **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** is designed to detect Influenza A virus (subtype H1N1) nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: nasal and oropharynx swabs and lavages.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use the **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit.**

The **Influenza A virus (subtype H1N1) 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 user manual.

2. METHOD

The implemented method of reverse transcription followed by polymerase chain reaction is based on RNA reverse transcription process and subsequent amplification of cDNA. 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 the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only when paraffin is melted or thermal dissociation of a complex of Taq polymerase and antibodies is happened. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) 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 PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the Influenza A virus (subtype H1N1) product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
Influenza A virus (subtype H1N1)	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** (see the catalogue at www.dna-technology.com to see available supply options).
The current version of the software is available for download at http://dna-technology.com/software.

3. CONTENT

The **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** contains RT-RANDOM primers and dNTP's, RT-buffer, reverse transcriptase, PCR-mix, PCR-buffer, Taq-polymerase, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** content, package S (standard) for R3-P408-23/4EU and R3-P408-S3/4EU

Reagent	Description	Total volume	Amount
Reverse transcription Kit			
RT-RANDOM primers and dNTP's	Colorless transparent liquid	50 µL	1 tube
RT-buffer	Colorless transparent liquid	100 µL	1 tube
Reverse transcriptase	Colorless transparent liquid	25 µL	1 tube
PCR detection kit			
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL per tube)	48 tubes or 6 8-tube strips
PCR-buffer	Colorless transparent liquid	500 µL	1 tube
Taq-polymerase	Colorless transparent viscous liquid	25 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	75 µL	1 tube
Strip's caps [1]	6 8-caps		

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

The kit is intended for single use and designed for 48 tests for **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit.**

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Specimen collection swabs: use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts;
- Use "DNA-Technology" made transport media **STOR-F** (REF P-901-1/1EU) or equivalent or sterile saline or sterile PBS for the transportation of the sample.

4.2. RNA extraction and PCR

Specimen and control preparation and reverse transcription area

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;

[1] - for detection kit packaged in strips REF R3-P408-S3/4EU

- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU) **DNA/RNA Extraction Kit** is recommended**;**
- High speed centrifuge (RCF 16000 x g);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 0.5 mL and 1.5 mL tubes;
- 0.5 mL and 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile) for the preparation of negative control sample (if needed);
- Container for used pipette tips;
- Single channel pipettes (dispensers covering 0.5 -1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL, 200 µL, 1000 µL);
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for strips (using detection kit packaged in strips REF R3-P408-S3/4EU);
- Refrigerator;
- PCR tube rack for 0.2 mL tubes in 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);
- Powder-free surgical gloves;
- Disinfectant solution;
- Container for used pipette tips.

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. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit,** except the Taq-polymerase, PCR-buffer and Reverse transcription Kit, must be stored at temperatures from 2 °C to 8 °C over the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance. The Taq-polymerase, PCR-buffer and Reverse transcription Kit must be stored at temperatures from minus 18°C to minus 22 °C during the storage period.

⚠ It is allowed the multiple freeze-thaw cycles for PCR-buffer and mineral oil.

The kit can be transported by all types of roofed transport at temperatures corresponding to the storage conditions of the kit components over the transportation. Transportation is allowed in thermal containers with icepacks by all types of covered transport at a temperatures up to 25 °C inside the container, but for no longer than 5 days.

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

- components of the kit, except the Taq-polymerase, PCR-buffer and Reverse transcription Kit, should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- Taq-polymerase, PCR-buffer and Reverse transcription Kit should be stored at temperatures from minus 18 to minus 22 °C during the storage period.

The kit stored in under undue regime should not be used.

An expired the **Influenza A virus (subtype H1N1) 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 virus (subtype H1N1) 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 **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit**.

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. Use powder-free surgical gloves. Use 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, 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 DNA 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 Master 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 **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** is designed to detect RNA extracted from the nasal cavity and pharynx swabs and lavages, depending on professional prescription.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the RNA extraction kit from biological material.

General requirements

The quality of taking a sample of biomaterial, its storage, transportation and pre-processing have a great importance for obtaining correct results. PCR research is a direct method, so taking of biological material must be carried out from the location of the infectious process.

Sample collection and preparation

Swabs from the nasal cavity

Take the swab with a dry sterile disposable probe into 1.5 mL plastic tubes with 300 mL of sterile saline solution or a 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 probe for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the probe from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used probe.
5. Close the tube tightly and mark it.

Swabs from the oropharynx

Take the swab with a dry sterile disposable probe into 1.5 mL plastic tubes with 300 mL of sterile saline solution or a 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 the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used probe.
5. Close the tube tightly and mark it.

Oropharyngeal lavage

Before taking oropharyngeal lavage, rinsing the mouth with water should be done. After that, patient should rinse the oropharynx (for 10-15 seconds) with 8.0-10 mL of sterile saline solution. Collect the liquid through a funnel into a sterile tube. Do not reuse the funnel without preliminary autoclaving. Transfer the lavage from the oropharynx (300 µL) into 1.5 mL plastic test tubes, close the test tube and mark.

Nasal cavity lavage

Take the material in the patient's sitting position with the head tilted back. To obtain a nasal cavity lavage, inject 3.0-5.0 mL of warm sterile saline solution alternately into both nasal passages using a probe or a disposable syringe. Collect the liquid through a funnel into a sterile tube. Do not reuse the funnel without preliminary autoclaving. Transfer the lavage from the oropharynx (300 µL) into 1.5 mL plastic test tubes, close the test tube and mark.

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

Transportation and storage of the samples

Samples may be transported and stored in physiological saline at temperatures from 2 °C to 8° C no more than 24 hours prior to analysis. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for one month.

In case of usage transport media biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-NA DNA/RNA Extraction Kit** user manual.

8. PROCEDURE

RNA extracting from biological material

RNA extraction is carried out according to the extraction kit instructions. **PREP-NA DNA/RNA Extraction Kit** is recommended. It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of RNA from the corresponding types of biomaterial.

Do not perform centrifugation as a pretreatment of the nasal cavity and pharynx swabs taken into transport medium, and samples of lavages.

Assay procedure

8.1 RNA extracting using PREP-NA DNA/RNA Extraction Kit

The lysis buffer supplied with **PREP-NA DNA/RNA Extraction Kit** can contain the precipitate. Dissolve it at 65 °C for 10 min prior to use.

At this step of assay use only RNase and DNase free pipette tips.

Independently of RNA extraction kit used, a negative control sample should go through all stages of RNA extraction. Physiological saline solution can be used as a negative control sample in volumes as indicated.

8.1.1 Mark the required number of 1.5 mL tubes for each sample to be tested and for negative control.

8.1.2 Add 300 µL of the lysis buffer into the each test tube and “C-“ tube.

8.1.3 Vortex the tube with analyzed material and spin down the drops for 3-5 seconds.

Open the tube, add sample, then close the tube before proceeding to the next sample to prevent contamination.

8.1.4 Add 100 µL of the sample into the marked tubes. Do not add samples to the “C-“ tube.

8.1.5 Add 100 µL of the negative control sample into corresponding tube.

8.1.6 Close the tubes tightly, vortex for 3-5 seconds and centrifuge for 3-5 seconds.

8.1.7 Thermostate the tubes for 15 min at 65 °C, spin down the drops at 16000 x g for 30 seconds.

8.1.8 Add 400 µL of the precipitation buffer. Close the tubes tightly and vortex them for 3–5 seconds.

8.1.9 Centrifuge the tubes at 16000 x g for 15 min.

8.1.10 Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each tube.

8.1.11 Add 500 µL of the washout solution №1 to the precipitate, close the tubes and mix by inverting the tubes 3-5 times.

8.1.12 Centrifuge the tubes at 16000 x g for 5 min.

8.1.13 Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each tube.

8.1.14 Add 300 µL of the washout solution №2 to the precipitate, close the tubes and mix by inverting the tube 3-5 times.

8.1.15 Centrifuge the tubes at 16000 x g for 5 min.

8.1.16 Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each tube.

8.1.17 Open the tubes and dry the precipitate at 65 °C for 5 min.

8.1.18 Add 50 µL of the dilution buffer to pellet, close the tubes and warm up the tubes at 65 °C for 10 min. Vortex the tubes for 3–5 seconds.

8.1.19 Centrifuge the tubes at 16000 x g for 30 seconds at room temperature.

The DNA/RNA preparation is ready for reverse transcription reaction.

The obtained RNA preparation is recommended to be used immediately for reverse transcription reaction.

8.2 Preparing reverse transcription

8.2.1 Mark the required number of 0.5 mL tubes for each sample to be tested and for negative control.

8.2.2 Unfreeze content of RT-buffer and RT-RANDOM primers and dNTP's tubes from Reverse transcription Kit at room temperature, then vortex thoroughly and spin down drops by centrifuging on vortex-microcentrifuge for 3-5 sec.

The RT-buffer supplied with Reverse transcription Kit can contain the precipitate. Dissolve it at 18-25 °C prior to use.

8.2.3 Prepare RT-mix. Add to the one tube:

- 2.0 x (N+1) µL of RT-buffer,
- 1.0 x (N+1) µL of RT-RANDOM primers and dNTP's,
- 0.5 x (N+1) µL of Reverse transcriptase,

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

Example: to test 5 samples, mark 5 tubes for samples and 1 tube for “C-”, prepare the RT-mix for 7 (6+1) tubes. Mix 14 µL of RT-buffer, 7.0 µL of primers and 3.5 µL of reverse transcriptase.

Reverse transcriptase should be kept out of freezer for as short time as possible.

8.2.4 Vortex RT-mix and spin down drops by centrifuging on vortex-microcentrifuge for 3-5 seconds.

8.2.5 Add 3.5 µL of RT-mix into each tube.

At this step of assay use only RNase and DNase free filtered pipette tips to prevent contamination.

8.2.6 Add 16.5 µL of corresponding RNA sample, using new tip for each sample. Do not add RNA in negative control tube.

8.2.7 Add 16.5 µL of negative control which passed all steps of RNA extraction procedure in negative control tube.

8.2.8 Vortex the tubes thoroughly (3-5 seconds) and spin briefly (1-3 seconds).

8.2.9 Place tubes in thermostat and incubate at 40 °C for 30 min, then heat up to 95 °C and leave for 5 min.

Use “DNA-Technology” Gnom Programmable thermostat or similar thermostats with clamping cover.

8.2.10 Spin the tubes at 16000 x g for 30 seconds to collect the drops.

The cDNA sample is ready for use in PCR.

The frozen cDNA material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for one month. For PCR, cDNA samples stored at temperatures from minus 18 °C to minus 22 °C must be unfrozen at room temperature or at temperatures from 2 °C to 8 °C.

8.3 Preparing PCR

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

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

8.3.1 Mark the required number of tubes with paraffin sealed PCR-Mix for each test sample, positive control (C+) and negative control (C-).

Example: to test 5 samples, mark 5 tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 7.

8.3.2 Vortex the PCR-buffer and Taq-polymerase thoroughly (3-5 seconds), then spin briefly (1-3 seconds) at room temperature (18–25 °C).

Taq-polymerase must be stored at temperatures from minus 18 °C to minus 22 °C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.3.3 Prepare Taq-polymerase solution. Add into the one tube:

- 10x(N+1) µL of PCR-Buffer,
- 0.5x(N+1) µL of Taq-polymerase,

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

Example: for simultaneous testing of 5 samples, “C-” and “C+” in one PCR run, mark 7 tubes (5 tubes for samples to be tested, 1 tube for “C+” and 1 tube for “C-”). Prepare the mixture of PCR-buffer and Taq-polymerase for 8 (7+1) tubes. Mix 80 µL of PCR-buffer and 4.0 µL of Taq-polymerase.

8.3.4 Vortex the tube with Taq-polymerase solution thoroughly (3-5 seconds) and spin briefly on vortex-microcentrifuge (1-3 seconds).

8.3.5 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

8.3.6 Add one drop (~20 µL) of mineral oil into each tube. Close tubes tightly.

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

Open the tube, add cDNA sample (or control sample), then close the tube before proceeding to the next cDNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next cDNA sample to prevent contamination. Use filter tips.

8.3.8 Add 5.0 µL of cDNA sample into corresponding tubes. Do not add cDNA into the “C+”, “C-“ strips. Avoid paraffin layer break. Close the tubes/strips tightly.

8.3.9 Add 5.0 µL of negative control (C-) which passed whole RNA extraction procedure and reverse transcription into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break. Close the tubes/strips tightly.

8.3.10 Spin tubes/strips for 1-3 seconds.

8.3.11 Set the tubes into the Real-time Thermal Cycler.

8.3.12 Launch the RealTime_PCR application in “Device operation” mode. Upload the .ini file supplied with the kit before first run. Please refer to DTlite or DTprime thermal cycler’s user manual for details on working with .ini files. In subsequent runs add corresponding test to the protocol, specify the number and ID’s of the samples, specify the position of the tubes/strips in the thermal unit (p. 8.3.11) and run PCR. See table 3.

Selecting a test, Table 3 should be displayed in the “Start run” window.

Table 3. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		v	
3	94	0	10	45		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10	…	…	Holding		Holding

9. CONTROLS

The **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the Influenza A virus (subtype H1N1) genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The PCR-mix from the kit includes the Internal control (IC). IC is an artificial plasmid intended to assess PCR performance. To reveal possible contamination a negative control is required.

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

The test result is considered valid when:

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

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

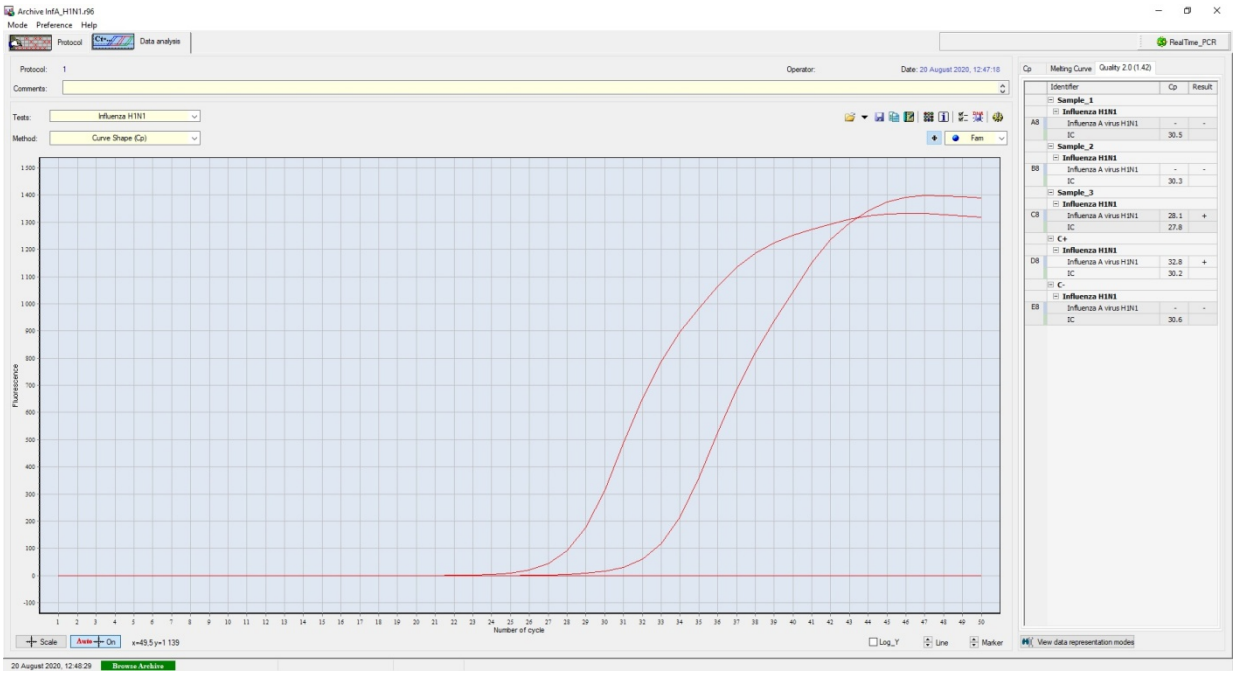
If positive control (C+) does **not** express growing fluorescence in the specific product or positive result, it is required to repeat the whole tests. It may be caused by inhibitors, operation error or violation of storage and handling.

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

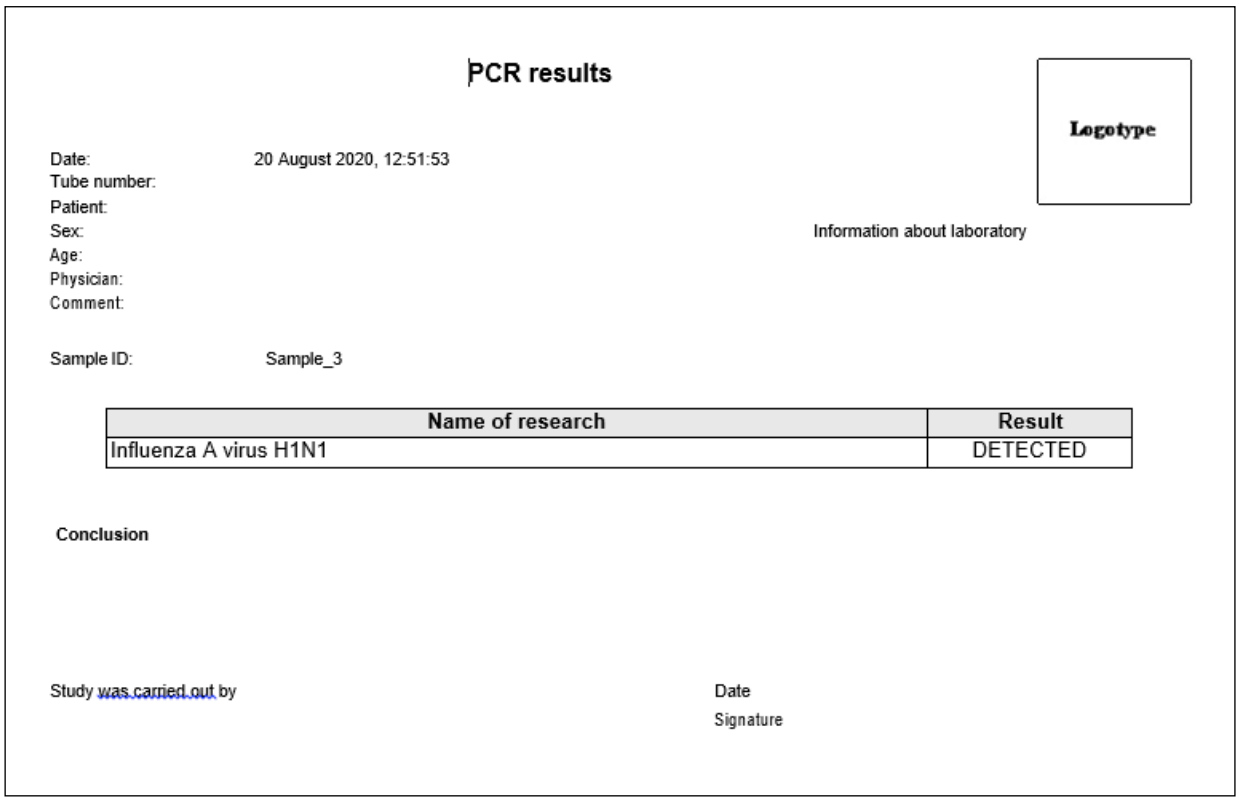
10. DATA ANALYSIS

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

Registration and interpretation of the PCR results are held in automatic mode. Analysis will be performed by Real-Time PCR application. The resulting graph will display the dependence of fluorescence intensity on the cycle number for each tube. Type of the sample, name of the test, value of the threshold cycle (Cp) and test result (qualitative) will be displayed in the right module of the window.



Operator can create, save and print a report.



PCR results

Logotype

Date: 20 August 2020, 12:51:53
Tube number:
Patient:
Sex:
Age:
Physician:
Comment:

Information about laboratory

Sample ID: Sample_3

Name of research	Result
Influenza A virus H1N1	DETECTED

Conclusion

Study was carried out by

Date
Signature

The interpretation should be performed in accordance with Table 4.

Table 4. Interpretation of PCR results

Fam	Hex (IC)	Result	Interpretation
Analyzed samples			
Cp is specified	Is not considered	“+”	Specific RNA is detected
Cp is not specified	Cp is specified	“-”	Specific RNA isn’t detected
Cp is not specified	Cp is not specified	Invalid	Invalid
“C+”			
Cp is specified	Cp is specified	“+”	Positive
“C-”			
Cp is not specified	Cp is specified	“-”	Negative

In the samples containing Influenza A virus (subtype H1N1) RNA (specific product), the Real-Time PCR Thermal Cycler registers the expressed growing fluorescence in specific product, the amplification result of the internal control is not taken into account.

In the samples free of Influenza A virus (subtype H1N1) RNA, the Real-Time PCR Thermal Cycler registers the expressed growing fluorescence in the internal control and its absence in the specific product.

When the expressed growing fluorescence both in the specific product and the internal control are not seen, the result of amplification is considered as uncertain. It may be due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or RNA extraction, or collecting of clinical material are required to be repeated.

When the growing fluorescence is expressed in specific product of negative control (C-), the results of whole series are considered false. It is required to eliminate contamination.

11. SPECIFICATIONS

a. The analytical specificity of the **Influenza A virus (subtype H1N1) 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.

The samples with Influenza A virus (subtype H1N1) RNA are to be registered positive for specific product (a fragment of the Influenza A virus (subtype H1N1) genome). The samples free of Influenza A virus (subtype H1N1) RNA are to be registered negative for specific product and positive for internal control.

b. In a determination of analytical sensitivity, the **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Analytical sensitivity is no more than 500 cop/mL.

The claimed specifications are guaranteed when RNA extraction is performed with **PREP-NA DNA/RNA Extraction Kit** REF P-002/1EU.

12. TROUBLESHOOTING

Table 5. Troubleshooting

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

Phone/Fax: +7(495)640.17.71.

E-mail: hotline@dna-technology.ru, http://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 customer service with quality issues of **Influenza A virus (subtype H1N1) REAL-TIME PCR Detection Kit**:

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14. KEY TO SYMBOLS

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

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