AmpliSens® *Influenza virus* A-type-FRT PCR kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**CS+, C+$_{A\ H1N1}$, C+$_{A\ H3N2}$**	Positive controls of amplification
EC REP	Authorized representative in the European Community	**IC**	Internal control
⚠	Caution		

1. INTENDED USE

AmpliSens® *Influenza virus* A-type-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and typing of *Influenza virus* A (identification to subtypes H1N1 and H3N2) RNA in *Influenza virus* cultures and in clinical material containing *Influenza virus* A RNA (nasal and oropharyngeal swabs; sputum or nasopharyngeal or tracheal aspirate; and autopsy material) using real-time hybridization-fluorescence detection of amplified products.

NOTE: The results of RT PCR analysis are taken into account in complex diagnostics of disease.

NOTE: **AmpliSens® *Influenza virus* A-type-FRT** PCR kit is recommended for analysis of cDNA samples in which *Influenza virus* A RNA was detected in the course of clinical material and viruses cultures analysis using **AmpliSens® *Influenza virus* A/B-FRT** PCR kit

2. PRINCIPLE OF PCR DETECTION

Influenza virus A RNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *Influenza virus* A-type-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-rec (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *Influenza virus* A-type-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP). The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE	ROX
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1			
cDNA-target	IC cDNA	*Influenza virus* A H1 cDNA	*Influenza virus* A N1 cDNA
Target gene	Artificially synthesized sequence	Hemagglutinin of *Influenza virus* A/H1N1	Neuraminidase of *Influenza virus* A/H1N1
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2			
cDNA-target	IC cDNA	*Influenza virus* A/H3N2 cDNA	*Influenza virus* A/H3N2 cDNA
Target gene	Artificially synthesized sequence	Hemagglutinin of *Influenza virus* A/H3N2	Neuraminidase of *Influenza virus* A/H3N2

3. CONTENT

AmpliSens® *Influenza virus* A-type-FRT PCR kit is produced in 1 form:
variant FRT-100 F, REF R-V54-100-F(RG,iQ,Dt,SC)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1	clear liquid from colorless to light lilac colour	0.12	10 tubes
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2	clear liquid from colorless to light lilac colour	0.12	10 tubes
PCR-mix-2-FRT	colorless clear liquid	0.6	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	2 tubes
Positive Control cDNA *Influenza virus* A H1N1 (C+$_{A\ H1N1}$)	colorless clear liquid	0.1	2 tubes
Positive Control cDNA *Influenza virus* A H3N2 (C+$_{A\ H3N2}$)	colorless clear liquid	0.1	2 tubes
TE-buffer	colorless clear liquid	1.0	1 tube
Positive Control STI (CS+)	colorless clear liquid	0.1	2 tubes
Negative Control (C–)*	colorless clear liquid	1.2	2 tubes
Internal Control STI-rec (IC)**	colorless clear liquid	0.12	10 tubes

* must be used in the extraction procedure as Negative control of extraction.

** add **10 µl** of **Internal Control STI-rec (IC)** during the RNA extraction procedure directly to the sample/lysis mixture (see **RIBO-prep**, REF K2-9-Et-100-CE protocol or **RIBO-sorb**, REF K2-1-Et-100-CE protocol.

Variant FRT-100 F is intended for 100 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium for storage and transportation of respiratory swabs.
- Reagent for pretreatment of viscous fluids (sputum, aspirates).

For DNA/RNA extraction, reverse transcription and amplification

- RNA extraction kit or the RNA extraction automatic station.
- Reverse transcription kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); SmartCycler II (Cepheid, USA) or equivalent).
- Disposable polypropylene PCR tubes:
  a) 0.2-ml PCR tubes (flat caps, nonstriped) if a rotor-type instrument is used.
  b) 0.025-ml microtubes (Cepheid, USA) if SmartCycler II instrument is used.
- Refrigerator at the temperature from 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Influenza virus* A-type-FRT PCR kit is intended for analysis of RNA extracted with RNA extraction kits from clinical material (nasal and oropharyngeal swabs; sputum or nasopharyngeal or tracheal aspirate; and autopsy material (fragments of damaged lungs)).

Sampling

Nasal swab samples are obtained using sterile dry flocked swabs with plastic shafts for nasopharyngeal swabs. If the nasal cavity is full of mucus it is recommended to blow the nose before the procedure. Gently insert the swab along the external nasal wall to a depth of 2–3 cm towards the inferior nasal concha. Then move the swab slightly lower, insert it in the inferior nasal meatus under the inferior nasal concha, rotate, and remove along the external nasal wall.

When the material is obtained, insert the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE). Break off the end of shaft to allow tight closing of the tube cap. Close the tube with the solution and the swab.

Oropharyngeal swab samples are obtained using sterile dry rayon swabs with plastic shafts for oropharyngeal swabs. Rotate the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx after gargling the oral cavity with water.

When material is obtained, insert the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE). Break off the end of shaft to allow tight closing of tube cap. Close the tube with the solution and the swab.

NOTE: It is recommended to combine nasal and oropharyngeal swabs in a single tube. For this purpose, place the ends of both shafts into one tube containing 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE) and analyze them as a single sample.

Sputum or nasopharyngeal or tracheal aspirate

Gargle the oral cavity with water. Collect sputum into a sterile disposable container.

Collect *nasopharyngeal or tracheal aspirate* by the conventional procedure and transfer them into the sterile disposable containers.

The samples can be stored before the analysis at 2–8 °C for 1 day, at ≤ –16 °C for 1 week.

Autopsy material

Collect autopsy material into sterile disposable containers and freeze immediately or analyze within 1 h. The samples can be stored at ≤ –68 °C for 1 year.

NOTE: Only one freeze-thaw cycle of clinical material is allowed.

Pretreatment

Nasal and oropharyngeal swabs

Vortex the tube, then centrifuge it at 5,000 rpm for 5 s to sediment drops from the cap.

Sputum or aspirate

Pretreat sputum or aspirate according to **Mucolysin** (REF 180-CE) *Instruction manual*. Use 100 µl of pretreated material for RNA extraction. Freeze the rest of sputum if it is necessary to repeat analysis later.

Autopsy material

Homogenize the samples using sterile porcelain mortars and pestles and then prepare 10 % suspension in sterile saline or phosphate buffer. Transfer the suspension into a 1.5-ml tube and centrifuge at 10,000 rpm for 5 min. Use 100 µl of the supernatant for RNA extraction. Freeze the rest of suspension if it is necessary to repeat analysis later.

7. WORKING CONDITIONS

AmpliSens® *Influenza virus* A-type-FRT PCR kit should be used at 18–25 °C

8. PROTOCOL

8.1. RNA Extraction

It is recommended to use the following nucleic acid extraction kits:

— **RIBO-prep**, REF K2-9-Et-50-CE; REF K2-9-Et-100-CE.

— **RIBO-sorb**, REF K2-1-Et-50-CE, REF K2-1-Et-100-CE.

— NucliSENS easyMAG automated system (for details see Guidelines [2]).

The RNA extraction of each clinical sample is carried out in the presence of **Internal Control STI-rec (IC).**

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– — Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

NOTE: Extract RNA according to the manufacturer's protocols.

NOTE: In case of extracting with the **RIBO-sorb** or **RIBO-prep** reagent kits the volume of the **Internal Control STI-rec (IC)** reagent added to each tube is **10 µl**.

NOTE: In case of extracting with the **RIBO-sorb** reagent kit, make sure that there are no suspended particles in the tubes before adding the sorbent. Otherwise, centrifuge the tubes at 10,000 rpm for 1 min and then transfer the supernatant to new tubes.

8.2. Reverse transcription

It is recommended to use the following kit for the complementary DNA (cDNA) synthesis from the RNA:

— **REVERTA-L**, REF K3-4-50-CE; REF K3-4-100-CE.

NOTE: Carry out the reverse transcription according to the manufacturer's protocols.

8.3. Preparing PCR

NOTE: At the amplification step, the positive controls (see Table 2), CS+, and NCA (intended for control of reagent purity and carefulness of operator's work) are used in every experiment. C– is also tested at the amplification step.

Table 2

Compliance of names of PCR-mixes-1-FEP/FRT and positive controls of amplification

PCR-mix-1-FEP/FRT	Positive control (C+)
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1	Positive Control cDNA *Influenza virus* A H1N1 ($C+_{A\ H1N1}$)
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2	Positive Control cDNA *Influenza virus* A H3N2 ($C+_{A\ H3N2}$)

8.3.1. Preparing tubes for PCR

The type of tubes depends on the type of PCR real-time instrument.

Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

1. Thaw the tubes with **PCR-mix-2-FRT, polymerase (TaqF),** and **PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1** (or **PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2**). Mix **PCR-mix-2-FRT, polymerase (TaqF),** and **PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1** (or **PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2**), then centrifuge briefly.
2. Prepare the required number of tubes. For N reactions (including controls), add to a new tube:
   - **10*(N+1) µl** of **PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1** (or **PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2**),
   - **5.0*(N+1) µl** of **PCR-mix-2-FRT**,
   - **0.5*(N+1) µl** of **polymerase (TaqF)**.

   Vortex the tube and centrifuge it briefly.
3. Transfer **15 µl** of the prepared mixture into each tube.
4. Add **10 µl** of **cDNA samples** obtained at the reverse transcription stage into the prepared tubes.
5. Carry out the control amplification reactions (for each PCR-mix-1-FEP/FRT):

NCA – Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative control of amplification).

$C+_{A\ H1N1}$ – Add **10 µl** of **Positive Control cDNA *Influenza virus* A H1N1 ($C+_{A\ H1N1}$)** to the tube labeled $C+_{A\ H1N1}$ (Positive control of amplification).

$C+_{A\ H3N2}$ – Add **10 µl** of **Positive Control cDNA *Influenza virus* A H3N2 ($C+_{A\ H3N2}$)** to the tube labeled $C+_{A\ H3N2}$ (Positive control of amplification).

CS+ – Add **10 µl** of **Positive Control STI (CS+)** to the tube labeled CS+ (Positive control of amplification of internal control).

C– – Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

6. When working with Smart Cycler II instrument, sediment the reaction mixture using the minicentrifuge provided with the instrument.

8.3.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 3

Amplification program for *Influenza virus* A H1N1 and A H3N2 cDNA

	Rotor-type Instruments[1]			Plate-type Instruments [2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**900 s**	**1**
2	**95**	**10 s**	**10**	**95**	**15 s**	**42**
	54	**20 s**				
	72	**10 s**				
3	**95**	**10 s**	**35**	**54**	**25 s *Fluorescent signal detection***	
	54	**20 s *Fluorescent signal detection***				
	72	**10 s**		**72**	**25 s**	

Fluorescent signal is detected in the channels for FAM, JOE, and ROX fluorophores.

NOTE: Amplification programs for the concrete model of the thermocycler are described in the Guidelines

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.

NOTE: Do not carry out reactions of identification to subtypes H1N1 and H3N2 simultaneously.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used. Results are interpreted by the crossing (or not-crossing) the fluorescence curve for each channel (according to Table 4) with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.

Table 4

Channels for detection of gene targets

PCR-mix-1-FEP/FRT	Detection in the channel for the fluorophore		
	FAM	**JOE**	**ROX**
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1	IC and CS+	*Influenza virus* A H1	*Influenza virus* A N1
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1	IC and CS+	*Influenza virus* A H3	*Influenza virus* A N2

Principle of interpretation is the following:

– The required fragment of the gene target is **detected** in the sample if its *Ct* value is determined in the results grid in the channel for pathogen detection.

– The required fragment of the gene target is **not detected** in the sample if its *Ct* value is not determined in the results grid (the fluorescence curve does not cross the threshold line) in the channel for pathogen detection and if the *Ct* value determined in the results grid in the channel for FAM fluorophore does not exceed the specified boundary value.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5).

Table 5

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore		
		FAM	**JOE**	**ROX**
		IC detection	**H1/H3 detection**	**N1/N2 detection**
C–	RNA extraction	< boundary value	Absent	Absent
NCA	PCR	Absent	Absent	Absent
CS+	PCR	< boundary value	Absent	Absent
$C+_{A\ H1N1}$, $C+_{A\ H3N2}$	PCR	Absent	< boundary value	< boundary value

[1] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia).

[2] For example, Smart Cycler II (Cepheid, USA).

- The sample is considered to be **positive** for A/H1 (or A/H3) if the *Ct* value is determined in the results grid in the channel for JOE fluorophore.
- The sample is considered to be **positive** for A/N1 (or A/N2) if the *Ct* value is determined in the results grid in the channel for ROX fluorophore.
- The sample is considered to be **negative** for A/H1N1 (or A/H3N2) subtype if the *Ct* value is not determined in the results grid in the channel for JOE and/or ROX fluorophores and if the *Ct* value determined in the results grid in the channel for FAM fluorophore does not exceed the specified boundary value.
- The result is considered to be **invalid** if the *Ct* value of a sample in the channel for JOE and ROX fluorophores is absent and the *Ct* value in the channel for FAM fluorophore is absent as well or exceed specified boundary value. It is necessary to repeat the PCR test for such a sample, starting from the RNA extraction stage. If the result is the same, repeat material sampling.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the channels for detection of some gene target is greater than the boundary *Ct* value or absent, the analysis should be repeated for all clinical samples which are negative in given channel.
2. If the *Ct* value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channels for detection of some gene target, the analysis (beginning with the RNA extraction stage) should be repeated for all samples in which given gene target was detected in order to exclude the consequence of possible contamination.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Influenza virus* A-type-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Influenza virus* A-type-FRT** PCR kit (except for PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1, PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2, polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Influenza virus* A-type-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1, PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2, Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use

NOTE: PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1 and PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2 are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Table 8

Clinical material	Nucleic acid extraction kit	Transport medium	Sensitivity, copies/ml
Nasal and oropharyngeal swabs	**RIBO-sorb**	**Transport Medium for Storage and Transportation of Respiratory Swabs** REF 959-CE; REF 957-CE; REF 958-CE	$1x10^3$
Nasal and oropharyngeal swabs	**RIBO-prep**	**Transport Medium for Storage and Transportation of Respiratory Swabs** REF 959-CE; REF 957-CE; REF 958-CE	$1x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® *Influenza virus* A-type-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.

AmpliSens® *Influenza virus* A-type-FRT PCR kit allows detection of fragments of hemagglutinin and neuraminidase genes encoding *Influenza virus* A/H1N1 and A/H3N2 strains.

Specific activity is confirmed by analysis of reference strains, 26 *Influenza virus* A/H1N1 isolates, and 23 *Influenza virus* A/H3N2 isolates extracted in 1977 to 2008 in Russian Federation, Ukraine and Belorussia. It was also confirmed for clinical material by sequence analysis of amplified fragments.

The activity was absent while testing fragments of hemagglutinin and neuraminidase genes of *Influenza virus* A subtypes H13, H9, H8N4, H2N3, H2N9, N8, H4N6, H11N6, H12N5, H6, H10N7, H5N3, H7, H5, and H5N3, *Influenza virus* B lineages Yamagata and Victoria; cDNA/DNA of strains and isolates of the main pathogens causing acute respiratory diseases as well as normal microflora of human nasal cavity and oropharynx; and human cDNA/DNA.

Positive reaction for hemagglutinin type 1 and negative reaction for neuraminidase type 1 were observed with PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1 while testing *Influenza virus* A/H1N1(sw2009) (A/California/04/2009(H1N1)). This means that *Influenza virus* A H1N1 is not detected.

The clinical specificity of **AmpliSens® *Influenza virus* A-type-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to **AmpliSens® *Influenza virus* A-type-FRT** PCR kit for qualitative detection and typing of *Influenza virus* A (identification to subtypes H1N1 and H3N2) RNA in *Influenza virus* cultures and in clinical material containing *Influenza virus* A RNA by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Influenza virus* A-type-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
27.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
18.03.15 ME	Text	Corrections according to the template. Grammar corrections
	6. Sampling and handling	Reference numbers for Transport Medium for Storage and Transportation of Respiratory Swabs was added
	8.1. RNA extraction	Additions about carrying out the control of extraction
	8.3.1. Preparing tubes for PCR	Additions about carrying out the negative control of extraction
	10. Troubleshooting	The section was rewritten
27.06.17 ME	6. Sampling and handling	In the procedure of nasal swabs sampling the probe with cotton swab was changed to flocked swabs with plastic shafts for nasopharyngeal swabs. In the procedure of oropharyngeal swabs sampling the probe with cotton swab was changed to rayon swabs with plastic shafts for oropharyngeal swabs
24.10.18 EM	3. Content	The colour of the reagents was specified
11.12.18 DV	2. Principle of PCR detection	The information about the enzyme UDG was added
09.10.19 PM	Through the text	Variant FRT (REF R-V54(RG)-CE; REF R-V54(iQ,Dt)-CE) was deleted. Corrections according to the template. The text formatting was changed
	2. Principle of PCR-detection	The table with target genes was added
04.06.20 MA	Footer	The phrase "Not for use in the Russian Federation" was added
11.03.21 MA	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP Ecoli Dx, s.r.o., Purkyňova 74/2
110 00 Praha 1, Czech Republic
Tel.: +420 325 209 912
Cell: +420 739 802 523

Federal Budget Institute of Science “Central Research Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia