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For Professional Use Only

AmpliSens® *Influenza virus* A-type-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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) by using real-time hybridization-fluorescence detection.

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

The AmpliSens® *Influenza virus* A-type-FRT PCR kit is recommended to analyze *Influenza virus* A RNA detected with the 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 real-time PCR, the amplified product is detected using 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 (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 separation of nucleotides and Taq-polymerase by using a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C. The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Influenza virus* A-type-FRT PCR kit is produced in 3 forms:

AmpliSens® *Influenza virus* A-type-FRT PCR kit variant FRT (for use with RG),

REF R-V54(RG)-CE;

AmpliSens® *Influenza virus* A-type-FRT PCR kit variant FRT (for use with iQ, Dt),

REF R-V54(iQ,Dt)-CE;

AmpliSens® *Influenza virus* A-type-FRT PCR kit variant FRT-100 F,

REF R-V54-100-F(RG,iQ,Dt,SC)-CE.

AmpliSens® *Influenza virus* A-type-FRT PCR kit variant FRT includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FEP/FRT *Influenza virus* A H1N1 (ready-to-use single-dose test tubes (*under wax)*)	colorless clear liquid	0.008	55 tubes of 0.2 ml
PCR-mix-1-FEP/FRT *Influenza virus* A H3N2 (ready-to-use single-dose test tubes (*under wax)*)	colorless clear liquid	0.008	55 tubes of 0.2 ml
PCR-mix-1-FL	colorless clear liquid	0.77	1 tube
Positive Control cDNA *Influenza virus* A H1N1 ($C+_{A\ H1N1}$)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *Influenza virus* A H3N2 ($C+_{A\ H3N2}$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Positive Control STI (CS+)	colorless clear liquid	0.1	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control STI-rec (IC)**	colorless clear liquid	0.12	5 tubes

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

** add 10 µl of Internal Control STI-rec during the RNA extraction procedure directly to the sample/lysis mixture (see extraction kit protocols).

AmpliSens® *Influenza virus* A-type-FRT PCR kit variant FRT is intended for 55 reactions (including controls).

AmpliSens® *Influenza virus* A-type-FRT PCR kit variant FRT-100 F includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1	colorless clear liquid	0.12	10 tubes
PCR-mix-1-FEP/FRT (F) *Influenza virus* A H3N2	colorless clear liquid	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-FL during the RNA extraction procedure directly to the sample/lysis mixture (see extraction kit protocols).

AmpliSens® *Influenza virus* A-type-FRT PCR kit variant FRT-100 F is intended for 100 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Transport medium.
- Mucolysin.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); SmartCycler II (Cepheid, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml, for example, Axygen, USA; or 0.025, for example, Cepheid, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.

- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area in which 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

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.

The material must be analyzed according to rules and instructions.

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

6.1. Material sampling

Nasal swabs

1. Take nasal swabs using a sterile swab with cotton pellet. If the nasal cavity is full of mucus it is recommended to blow the nose before the procedure. Gently insert the

swab with cotton pellet along the floor of the nasal passage into the nostril (to a depth of 2-3 cm from the inferior nasal concha). Turn the swab down and insert it into the lower nasal passage under the inferior nasal concha. Rotate the swab and remove it along the floor of the nasal passage.

2. Transfer the working part of the swab with cotton pellet to a tube with 500 µl of the transport medium for storage and transportation of respiratory swabs. Cut the swab handle so it does not protrude and the tube closes tightly. Close the tube containing the solution and the working part of the swab.

Oropharyngeal swabs

1. Gargle with water. Take throat swabs using sterile swab with cotton pellet by rotation from the tonsils area, palatoglossal arch, and the posterior nasopharynx.
2. Transfer the cotton pellet to a tube with 500 µl of the transport medium for storage and transportation of respiratory swabs. Cut the swab handle so it does not protrude and the tube closes tightly. Close the tube containing the solution and the working part of the swab.

It is recommended to collect throat and nasal swabs into the same tube with 500 µl of the transport medium for storage and transportation of respiratory swabs and analyze it as one sample.

Sputum nasopharyngeal or tracheal aspirate

Gargle with water. Collect sputum into a sterile container.

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

Before analysis, the samples are stored at 2–8 °C for 1 day, at ≤ –16 °C for 1 week.

Autopsy material

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

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

6.2. Preparation of samples

Nasal and oropharyngeal swabs

Vortex the tube, then centrifuge it at 5000 rpm for 5 s to sediment drops from the interior wall of the tube lid.

Sputum or aspirate

Prepare sputum or aspirate according to Mucolysin instruction. Use 100 µl for RNA extrac-

tion. Freeze the rest of aspirate 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 PBS. Transfer the suspension into a 1.5-ml tube and centrifuge at 10000 rpm for 5 min. Use 100 µl of the supernatant for RNA extraction. Freeze the rest of aspirate 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; K2-9-Et-100-CE.
- RIBO-sorb, **REF** K2-1-Et-50-CE, K2-1-Et-100-CE.
- The NucliSENS easyMAG automated nucleic acid extraction platform can be used as well.

Extract RNA according to the manufacturer's instructions.

Add **10 µl** of **Internal Control-FL** during the RNA extraction procedure directly to the sample/lysis mixture.

8.2. Reverse transcription

It is recommended that the following reverse transcription reagent kit is used:

- REVERTA-L, **REF** K3-4-50-CE.

Carry out the reverse transcription according to the manufacturer's instructions.

8.3. Preparing PCR

Use controls of amplification ($C+_{A\ H1N1,}$ $C+_{A\ H3N2}$, CS+, and NCA) and C– for each PCR-mix-1 every time when amplification reactions are carried out (see Table 1).

Table 1

Correspondence between PCR-mixes-1 and positive controls

PCR-mix-1	Positive Control cDNA
PCR-mix-1-FEP/FRT *Influenza virus* A H1N1, 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 *Influenza virus* A H3N2, 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

Variant FRT

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Influenza virus* A H1N1** (or **PCR-mix-1-FEP/FRT *Influenza virus* A H1N1**) for amplification of cDNA from clinical and control samples. Make sure that wax completely covers the solution at the bottom.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Influenza virus* A H1N1** (or **PCR-mix-1-FEP/FRT *Influenza virus* A H3N2**).
3. Add **10 µl** of **cDNA** obtained from clinical or control samples at the reverse transcription stage to the prepared tubes.
4. Carry out the control amplification reactions:

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** 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** to the tube labeled $C+_{A\ H3N2}$ (Positive control of amplification).

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

5. Vortex the mixture for 1-2 s in lower part of the tube (for plate-type Instruments).

Variant FRT-100 F

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 briefly.

3. Transfer **15 µl** of the prepared mixture to prepared tubes.

4. Add **10 µl** of c**DNA** obtained from clinical or control samples at the reverse transcription stage to the prepared tubes.

5. Carry out the control amplification reactions:

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** 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** to the tube labeled C+ $_{A\ H3N2}$ (Positive control of amplification).

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

6. Vortex the mixture for 1-2 s in lower part of the tube (for plate-type Instruments).

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

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

Table 2

Programming thermocyclers for amplification of *Influenza virus* A H1N1 and A H3N2 cDNA

	Rotor-type Instruments [1]		
Step	***Temperature, °C***	***Time***	***Cycles***
Hold	**95**	5 min	1
Cycling 1	**95**	10 s	10
	54	20 s	
	72	10 s	
Cycling 2	**95**	10 s	35
	54	20 s *fluorescent signal detection*	
	72	10 s	

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.

Table 3

Programming thermocyclers for amplification of *Influenza virus* A H1N1 and A H3N2 cDNA variant FRT-100 F

	Rotor-type Instruments [1]			Plate-type Instruments [2]		
Step	***Temperature, °C***	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
Hold	**95**	15 min	1	**95**	900 s	1
Cycling 1	**95**	10 s	10	**95**	15 s	42
	54	20 s				
	72	10 s				
Cycling 2	**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 designed for the FAM/Green, JOE/Yellow/Cy3, and ROX/Orange/Texas Red fluorophores at the stage Cycling 2 (54 °C).

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin.*

3. Insert tubes into the reaction module of the device.

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

See **Guidelines** for data analysis settings for the instrument.

Table 4

Channels for detection of gene targets

PCR-mix-1	Detection in channel		
	FAM/Green	JOE/Yellow/Cy3	ROX/Orange/Texas Red
PCR-mix-1-FEP/FRT *Influenza virus* A H1N1, 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 *Influenza virus* A H3N2, PCR-mix-1-FEP/FRT (F) *Influenza virus* A H1N1	IC and CS+	*Influenza virus* A H3	*Influenza virus* A N2

[2] For example, Smart Cycler II or equivalent.

1. 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.
2. 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 FAM/Green channel does not exceed the specified boundary value.

9.1. Interpretation of results

The results are interpreted by the software of the instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

Table 5

Results for controls

Control	Stage for control	Ct value in channels			Interpretation
		FAM/Green	JOE/Yellow/ Cy3	ROX/Orange/ Texas Red	
C–	RNA extraction	Pos (< boundary value*)	Neg	Neg	OK
NCA	Amplification	Neg	Neg	Neg	OK
CS+	Amplification	Pos (< boundary value*)	Neg	Neg	OK
C+$_{A\ H1N1}$, C+$_{A\ H3N2}$	Amplification	Neg	Pos (< boundary value*)	Pos (< boundary value*)	OK

* For boundary values, see the *Important Product Information Bulletin* and **Guidelines**.

1. The sample is considered to be **positive** for A/H1 (or A/H3) if its Ct value is determined in the results grid in the JOE/Yellow/Cy3 channel.
2. The sample is considered to be **positive** for A/N1 (or A/N2) if its Ct value is determined in the results grid in the ROX/Orange/Texas Red channel.
3. The sample is considered to be **negative** for A/H1N1 (or A/H3N2) subtype if its Ct value is not determined in the results grid (the fluorescence curve does not cross the threshold line) in JOE/Yellow/Cy3 and/or ROX/Orange/Texas Red channels and if the Ct value determined in the results grid in the FAM/Green channel does not exceed the specified boundary value.
4. The result is considered to be **invalid** if the Ct value of a sample in JOE/Yellow/Cy3 and ROX/Orange/Texas Red channels is absent and the Ct value in the FAM/Green channel is absent as well. 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.

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.

10. TROUBLESHOOTING

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

- If the Ct value is absent in JOE/Yellow/Cy3 and/or ROX/Orange/Texas Red channels or the Ct value in the FAM/Green channel is higher than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (if iCycler iQ or iQ5 instruments are used).
- If the Ct value is present for C– in JOE/Yellow/Cy3 and/or ROX/Orange/Texas Red channels and/or for NCA in all channels in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer's instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® *CMV*-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.

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.

PCR-mix-1-FEP/FRT *Influenza virus* A H1N1, PCR-mix-1-FEP/FRT *Influenza virus* A H3N2, 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

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

13.2. Specificity

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.

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

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 *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.

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, Moscow, 2008.
2. Guidelines to AmpliSens® *Influenza virus* A-type-FRT PCR kit.

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.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**CS+, $C+_{A\ H1N1}$, $C+_{A\ H3N2}$**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

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”