AmpliSens® *Influenza virus* A/H1-swine-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	C+	Positive control of amplification
EC REP	Authorized representative in the European Community	IC	Internal control
	Caution	CS+	Positive Control of Amplification of IC

1. INTENDED USE

AmpliSens® *Influenza virus* A/H1-swine-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Influenza virus* A/H1N1(sw2009) RNA in clinical materials (nasal and oropharyngeal swabs, sputum or nasopharyngeal and tracheal aspirates, and autopsy material (fragments of affected parts of lungs)) by using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

Influenza virus A/H1N1(sw2009)[1] detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Influenza virus* A/H1N1(sw2009) primers. In the 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 PCR 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/H1-swine-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/H1-swine-FRT PCR kit uses "hot-start," which greatly reduces the frequency of nonspecifically primed reactions. In variant FRT-50 F, "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 variant FRT-50 F 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
cDNA-target	IC cDNA	*Influenza virus* A/H1N1(sw2009) c DNA
Target gene	Artificially synthesized sequence	H1 gene of *Influenza virus* A type H1N1

3. CONTENT

AmpliSens® *Influenza virus* A/H1-swine-FRT PCR kit is produced in 2 forms:

variant FRT REF R-V55(RG)-CE.

variant FRT-50 F REF R-V55-F(SC)-CE.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT *Influenza virus* A/H1-swine (ready-to-use single-dose test tubes (*under wax)*)	clear liquid from colorless to light lilac colour	0.008	55 tubes of 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
Positive Control cDNA *Influenza virus* A/H1-swine (C+)	colorless clear liquid	0.1	1 tube
Positive Control STI-88 (CS+)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	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 (IC)** during the RNA extraction procedure directly to the sample/lysis mixture (see **RIBO-sorb,** REF K2-1-Et-50-CE, or **RIBO-prep**, REF K2-9-Et-50-CE, protocols).

Variant FRT is intended for 55 reactions (including controls).

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine	clear liquid from colorless to light lilac colour	0.12	5 tubes
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control cDNA *Influenza virus* A/H1-swine (C+)	colorless clear liquid	0.1	1 tube
Positive Control STI-88 (CS+)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	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 (IC)** during the RNA extraction procedure directly to the sample/lysis mixture (see **RIBO-sorb,** REF K2-1-Et-50-CE, or **RIBO-prep**, REF K2-9-Et-50-CE, protocols).

Variant FRT-50 F is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of respiratory swabs.
- RNA extraction kit or the RNA extraction automatic station.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol 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); Rotor-Gene Q (QIAGEN, Germany), SmartCycler II (Cepheid, USA), iCycler iQ or iQ5 (Bio-Rad, USA), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene tubes for PCR kit variant FRT-50 F:
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer for the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

[1] The name of the virus released by WHO is Influenza A virus (H1N1) pdm09.

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 positive material (specimens, controls) away from all other reagents and add it to the reaction mix in a distantly separated facility. Store and handle amplicons away from all other reagents.
- 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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all samples 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.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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/H1-swine-FRT PCR kit is intended for analysis of the RNA extracted with RNA extraction kits from the clinical material (nasal and oropharyngeal swabs; sputum (or nasopharyngeal and tracheal aspirates), autopsy material (fragments of affected parts of lungs)).

NOTE: It is recommended to combine nasal and oropharyngeal material in one tube. To do this, after sampling, place the working ends of swabs into one tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** and analyze as one sample.

Swabs are used without pretreatment.

Sputum or tracheal aspirate. Use reagent **Mucolysin** (REF 180-CE) manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology” for sputum pretreatment. See the *Instruction Manual* to **Mucolysin** for a proper use. The pretreated sputum (100 µl) is used for RNA extraction. If it is necessary to repeat the test, the rest of sputum can be frozen.

Autopsy material is homogenized using sterile porcelain mortars and pestles. Then, prepare a 10 % suspension in a sterile saline or phosphate buffer. Transfer the suspension to a 1.5-ml tube and centrifuge at 10,000 rpm for 5 min. The supernatant (100 µl) is used for RNA extraction. If it is necessary to repeat the test, the remaining sputum can be frozen.

7. WORKING CONDITIONS

AmpliSens® *Influenza virus* A/H1-swine-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-sorb**, REF K2-1-Et-50-CE,
- **RIBO-prep**, REF K2-9-Et-50-CE.
- NucliSENS easyMAG automated system (for details see Guidelines [2]).

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

In the extraction procedure for each panel 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 protocol.

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

8.2. Reverse transcription

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

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

NOTE: Carry out the reverse transcription according to the manufacturer’s instructions.

8.3. Preparing PCR

The total reaction volume is **25 µl**, the volume of cDNA sample is **10 µl**.

8.3.1. Preparing tubes for PCR

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

Variant FRT

1. Take the required number of tubes with **PCR-mix-1-FEP/FRT *Influenza virus* A/H1-swine** and wax for amplification of cDNA from clinical and control samples.
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/H1-swine**.

Variant FRT-50 F

1. Thaw the tubes with **PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine, PCR-mix-2-FRT,** and **polymerase (TaqF)**. Vortex the tubes, then centrifuge briefly.
   Take the required number of the tubes/stripes for amplification of cDNA obtained from clinical and control samples.
2. For N reactions, add to a new tube:
   10*(N+1) µl of **PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine**
   5.0*(N+1) µl of **PCR-mix-2-FRT;**
   0.5*(N+1) µl of **polymerase (TaqF).**
   Vortex the tube, then centrifuge it briefly. Transfer **15 µl** of the prepared mixture to each tube.

Steps 3 and 4 are required in both variants.

3. Add **10 µl** of c**DNA samples** obtained from clinical or control samples at the RNA reverse transcription stage into the prepared tubes using tips with aerosol barrier.
4. Carry out the control amplification reactions:

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

C+ – Add **10 µl** of **Positive Control cDNA *Influenza virus* A/H1-swine (C+)** to the tube labeled C+ (Positive Control of Amplification).

CS+ – Add **10 µl** of **Positive Control STI-88** to the tube labeled CS+ (Positive Control of Amplification of IC)

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

8.3.2. Amplification

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

Table 2

***Influenza virus* A/H1N1(sw2009) cDNA amplification program**

	Rotor-type instruments[2]			Plate-type instruments[3]		
Step	**Tempera-ture, °C**	**Time**	**Cycles**	**Tempera-ture, °C**	**Time**	**Cycles**
1	**95**	**5 min (variant FRT) 15 min (variant FRT-50 F)**	**1**	**95**	**5 min (variant FRT) 15 min (variant FRT-50 F)**	**1**
2	**95**	**10 s**	**10**	**95**	**10 s**	**10**
	54	**20 s**		**54**	**25 s**	
	72	**10 s**		**72**	**25 s**	
3	**95**	**10 s**	**35**	**95**	**10 s**	**35**
	54	**20 s *Fluorescent signal detection***		**54**	**25 s *Fluorescent signal detection***	
	72	**10 s**		**72**	**25 s**	

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

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.
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 by measuring fluorescence signal accumulation in two channels:

- The signal of the *Influenza virus* A/H1N1(sw2009)[4] RNA amplification product is detected in the channel for the JOE fluorophore,
- The signal of the Internal Control STI-rec cDNA amplification product is detected in the channel for the FAM fluorophore.

The results are interpreted by the crossing (or not-crossing) of the fluorescence curve 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.

Principle of interpretation is the following:

- *Influenza virus* A/H1N1(sw2009) RNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *Influenza virus* A/H1N1(sw2009) RNA is **not detected** if the *Ct* value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channel for the JOE fluorophore, whereas the *Ct* value determined in the results grid in the channel for the FAM fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.
- The result is **invalid** if the *Ct* value is not determined (absent) (the fluorescence curve does not cross the threshold line) in the channel for the JOE fluorophore, whereas the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or greater than the specified boundary *Ct* value. In such cases PCR should be repeated starting from the RNA extraction stage.

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 3).

Table 3

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**	
		FAM	**JOE**
C–	RNA extraction	< boundary value	Absent
NCA	PCR	Absent	Absent
C+	PCR	Absent	< boundary value
CS+	PCR	< boundary value	Absent

[2] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[3] For example, Mx3000P, Mx3000, iCycler iQ, iQ5, or equivalent.
[4] The name of the virus released by WHO is Influenza A virus (H1N1) pdm09.

10. TROUBLESHOOTING

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

1. If the *Ct* value for the Positive Control of Amplification (C+) in the channel for the JOE fluorophore is absent, the amplification and detection should be repeated for all negative samples.
2. If a *Ct* value is determined for Negative Control of Extraction (C–) in the channel for the JOE fluorophore and for the Negative Control of Amplification (NCA) in the channels for FAM and JOE fluorophores, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples.
3. If the *Ct* value for test samples in the channel for the JOE fluorophore is greater than the boundary *Ct* value specified in the *Important Product Information Bulletin,* the result is **equivocal**. The assay should be repeated in two replicates. If the reproducible *Ct* value is obtained, the result is positive; if reproducible *Ct* value is not obtained in two replicates – the result is **equivocal**.

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/H1-swine-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/H1-swine-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine, PCR-mix-2-FRT, and polymerase (TaqF)). All components of the **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit are stable until the expiry 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/H1-swine, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FEP/FRT *Influenza virus* A/H1-swine and PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit is following:

Table 4

Clinical material	Nucleic acid extraction kit	Sensitivity, copies/ml
Nasal and oropharyngeal swabs[5]	RIBO-prep	$1x10^3$
	RIBO-sorb	$5x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict 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/H1-swine-FRT PCR kit determines the fragment of the *Influenza virus* A/H1N1 hemagglutinin gene, which is related to the North American strain of *Influenza virus* swine. The specific activity of the PCR kit was confirmed by analyzing A/California/04/2009(H1N1) and A/California/07/2009(H1N1) isolates provided by CDC.

Nonspecific reactions were absent is tests of 26 reference strains and isolates of epidemic *Influenza viruses* A/H1N1 isolated in 1977-2008 in the Russian Federation, Ukraine, and Belarus Republics; *Influenza virus* A subtypes H13N2, H9N2, H8N4, H2N3, H2N9, H3N2, H3N8, H4N6, H11N6, H12N5, H1N1, H6N2, H10N7, H5N3, H7N1, H5N2, H5N3, and H2N2; *Influenza virus* B strains Yamagata and Victoria; as well as strains and isolates of the main pathogens causing human acute respiratory infections; and nucleic acids of the human genome.

The clinical specificity of **AmpliSens® *Influenza virus* A/H1-swine-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 State Institute of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.
2. Guidelines to the **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit "Studying of clinical material for the presence of *Influenza virus* A/H1N1 (sw2009) 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/H1-swine-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
24.01.11	Reference number	Reference numbers R-V55(iQ)-CE and R-V55-F(RG,iQ,Dt,SC)-CE are added
	Cover page	The phrase "For Professional Use Only" was added
	Content	New sections "Working Conditions" and "Transportation" were added
		The "Explanation of Symbols" section was renamed to "Key to Symbols Used"
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that PCR-mix-1-FEP/FRT *Influenza virus* A/H1-swine and PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine are to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
23.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
24.07.12 IvI	Content	AmpliSens® *Influenza virus* A/H1-swine-FRT PCR kit variant FRT-50 F (for use with SC) was added
	Footer	A new catalogue number was added
27.04.15 PM	Text	Corrections according to the template. Grammar corrections
	8.1. RNA extraction	Additions about carrying out the control of extraction
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
15.03.18 PM	Footer, 3.Content	REF R-V55(iQ)-CE was deleted
04.10.18 PM	14. References	The name of Guidelines was changed
11.12.18 DV	2. Principle of PCR detection	The information about the enzyme UDG was added
11.03.19 DV	3. Content	The colour of the reagent was specified
18.10.19 PM	Through the text	REF R-V55-F(RG,iQ,Dt,SC)-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. The link about the name of the virus was added
03.06.20 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®

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[5] Nasal and oropharyngeal swabs should be placed into **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 957-CE, REF 958-CE, REF 959-CE).