AmpliSens® *Influenza virus* A/B-FRT PCR kit

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+$_{A/B/STI}$**	Positive control of amplification
EC REP	Authorized representative in the European Community	**IC**	Internal control
	Caution		

1. INTENDED USE

AmpliSens® *Influenza virus* A/B-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of RNA of *Influenza virus* A and *Influenza virus* B in the biological material (nasopharyngeal and oropharyngeal swabs; sputum; aspirate of trachea; bronchoalveolar lavage, bronchial washing fluid, autopsy material, viral culture) 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 (fragment of M gene) and *Influenza virus* B (fragment of NS gene) detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Influenza virus* A and *Influenza virus* B 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/B-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/B-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 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
DNA-target	Internal Control cDNA	*Influenza virus* B cDNA	*Influenza virus* A cDNA
Target gene	Artificially synthesized sequence	NS gene of *Influenza virus* B	M1 gene of *Influenza virus* A

3. CONTENT

AmpliSens® *Influenza virus* A/B-FRT PCR kit is produced in 1 form:
variant FRT-100 F, REF R-V36-100-F-Mod(RG,iQ,Dt,CFX,SC)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL-F *Influenza virus* A/B	clear liquid from colorless to light lilac colour	0.2	5 tubes
PCR-mix-2-FRT	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Positive Control cDNA *Influenza virus* A / *Influenza virus* B / STI (C+$_{A/B/STI}$)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	2 tube
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-sorb,** REF K2-1-Et-50-CE protocol or **RIBO-prep,** REF K2-9-Et-50-CE protocol).

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

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of respiratory swabs.
- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- Flocked or fiber swabs for collecting specimens from kids and adults.
- 0.9 % saline solution or 0.01 M potassium-phosphate buffer (pH 7.0) for pretreatment of autopsy material or in case of viral cultures testing.
- RNA/DNA extraction kit or RNA/DNA extraction automatic station.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free 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.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany) iCycler iQ or iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA), SmartCycler II (provided with Mini-Spin centrifuge) (Cepheid, USA); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene 0.2- or 0.1-ml tubes for PCR:
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) thin-walled 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.
  c) special reaction modules for the SmartCycler II instrument.
- Refrigerator for 2–8 °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/B-FRT PCR kit is intended for analysis of RNA extracted with RNA/DNA extraction kits from the biological material (nasopharyngeal and oropharyngeal swabs; sputum; aspirate of trachea; bronchoalveolar lavage, bronchial washing fluid, autopsy material, viral culture).

Sampling

NOTE: It is recommended to combine nasopharyngeal and oropharyngeal swab samples in a single tube. To do this, collect each swabs by a different probe and place the effective part of both probes 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 as a single sample.

6.1 *Nasopharyngeal swab.* Before sampling, make the patient blow his nose if it is filled with mucus. The sample should be taken with a sterile flocked swab with a plastic applicator shaft. Gently insert the probe through the nostril 2-3 cm deep towards the inferior nasal concha. Lower the probe and pass it under the inferior nasal concha up to nasopharynx. Rotate and remove the swab. In total the probe should be inserted up to the half length from a nostril to an ear hole (3-4 cm in children and 5-6 cm in adults). Place the working part of the probe into a 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 probe, tightly secure the cap, and mark the tube.
It is possible to use a sterile polystyrene probe with a viscose tip in adults.

6.2 *Oropharyngeal swab* is taken with a sterile dry probe with a viscose tip. Rotate the probe over the tonsillar area, palatine arches, and posterior area of the pharynx. Place the working part of the probe (with the viscose tip) into the tube containing 0,5 ml of **Transport medium for storage and transportation of respiratory swabs** (REF 959-CE, REF 957-CE, REF 958-CE) and a nasal swab. Break off the probe, tightly secure the cap, and mark the tube.
Collected material can be stored at 2–8 °C for 3 days or at ≤ –16 °C for 1 week.

6.3 *Sputum or tracheal aspirate.* Sputum is collected into a sterile disposable plastic container. Before sampling, make the patient rinse his mouth with water. *Tracheal aspirate* is collected in accordance with the conventional technique and placed into a sterile disposable plastic container.
Collected material can be stored at 2–8 °C for 1 day or at ≤ –16 °C for 1 week.

6.4 *Bronchoalveolar lavage and bronchial washing fluid* are collected into a disposable, tightly screwed polypropylene container of at least 5-ml volume.
Collected material can be stored at 2–8 °C for 1 day or at ≤ –16 °C for 1 week.

6.5 *Autopsy material* should be placed into a sterile disposable container and analyzed within 1 hour otherwise it should be frozen.
Collected material can be stored at ≤ –16 °C for 1 week or at ≤ –68 °C for 1 year. Only one freeze–thaw cycle is allowed.

Pretreatment

6.6 *Respiratory swabs*. Vortex and then centrifuge closed tubes at 5,000 rpm for 5 s to remove drops from the tube walls. Collect 100 µl of a sample for RNA extraction.

6.7 *Sputum or tracheal aspirate.* To reduce viscosity of the material (if required), use a reagent for pretreatment of viscous fluids (for example **Mucolysin**, REF 180-CE, produced by FBIS CRIE) in accordance wtih manufacturer's instructions. The prepared sample (100 µl) is used for RNA extraction. The remained sample can be frozen for further use.

6.8 *Bronchoalveolar lavage and bronchial washing fluid.* Turn the container with the collected material upside down. Transfer 1ml of the sample into the 1.5-ml tube (use a tip with aerosole barrier) and centrifuge at 10,000 rpm for 10 min. Remove and discard the supernatant leaving 200 µl of fluid above the pellet (use a tip with aerosol barrier). Resuspend the pellet in the rest of the supernatant. Use 100 µl of the prepared suspension for RNA extraction. Freeze the remained suspension for further use.

6.9 *Autopsy material*. Homogenize the material with a sterile porcelain mortar and pestle. Then, prepare 10 % suspension in sterile saline or phosphate buffer. Transfer the suspension into a 1.5-ml tube, centrifuge at 10,000 rpm for 5 min, and use the supernatant (100 µl) for RNA extraction. The remained suspension can be frozen for further use.

6.10 *Viral cultures* should be used without pretreatment. Use 10 µl of culture fluid for RNA extraction.

7. WORKING CONDITIONS

AmpliSens® *Influenza virus* A/B-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 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: Carry the RNA extraction according to the manufacturer's protocol.

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

NOTE: If extracting with **RIBO-sorb** reagent kit, make sure that there aren't suspended particles in the tubes before adding sorbent. Otherwise, centrifuge the tubes at 10,000 rpm for 1 min and then transfer the supernatant in clean tubes.

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 protocol.

8.3. Preparing PCR

8.3.1 Preparing tubes for PCR

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

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

1. Thaw the required number of tubes with **PCR-mix-1-FL-F *Influenza virus* A/B**. Vortex the tubes with **PCR-mix-1-FL-F *Influenza virus* A/B, PCR-mix-2-FRT,** and **polymerase (TaqF)** and then centrifuge briefly.
   Take the required number of the tubes/strips 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-FL-F *Influenza virus* A/B,
   5.0*(N+1) µl of PCR-mix-2-FRT
   0.5*(N+1) µl of polymerase (TaqF).
   Vortex the tube, then centrifuge briefly. Transfer **15 µl** of the prepared mixture to each tube.

Table 2

Scheme of reaction mixture preparation for variant FRT-100 F

	Reagent volume for specified number of reactions		
Reagent volume per one reaction, µl	**10.0**	**5.0**	**0.5**
Number of reactions[1]	**PCR-mix-1-FL-F *Influenza virus* A/B**	**PCR-mix-2-FRT**	**Polymerase(TaqF)**
6	**60**	**30**	**3.0**
8	**80**	**40**	**4.0**
10	**100**	**50**	**5.0**
12	**120**	**60**	**6.0**
14	**140**	**70**	**7.0**
16	**160**	**80**	**8.0**
18	**180**	**90**	**9.0**
20	**200**	**100**	**10.0**
22	**220**	**110**	**11.0**
24	**240**	**120**	**12.0**
26	**260**	**130**	**13.0**
28	**280**	**140**	**14.0**
30	**300**	**150**	**15.0**
32	**320**	**160**	**16.0**

3. Using tips with aerosol filter, add **10 µl of cDNA samples** obtained at the RNA reverse transcription stage.
4. Carry out the control amplification reactions:

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

$C+_{A/B/STI}$ – Add **10 µl** of **Positive Control cDNA *Influenza virus* A / *Influenza virus* B / STI ($C+_{A/B/STI}$)** to the tube labeled $C+_{A/B/STI}$

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

8.3.2. Amplification

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

Table 3

Amplification program for *Influenza virus* A/B cDNA

Step	Rotor-type instruments[2]			Plate-type instruments[3]		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	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 Fluorescence acquiring		54	25 s Fluorescence acquiring	
	72	10 s		72	25 s	

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

Table 4

Amplification program for *Influenza virus* A/B cDNA for SmartCycler (Cepheid, USA) instrument

Step	Temperature, °C	Time	Cycles
Hold	95	900 s	1
Stage1	95	15 s	42
	54	25 s Fluorescence acquiring	
	72	25 s	

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

Amplification program for some other models of thermocyclers are specified in Guidelines [2].

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

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.

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

[1] Number of test samples including the control of extraction stage (N), controls of amplification, and one extra reaction (N+3+1).
[2] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene (QIAGEN, Germany).
[3] For example, iCycler iQ, iQ5 (Bio-Rad, USA).

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels:

– The signal of the IC cDNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *Influenza virus* B cDNA amplification product is detected in the channel for the JOE fluorophore.
– The signal of the *Influenza virus* A cDNA amplification product is detected in the channel for the ROX fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at a specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the result grid.

Principle of interpretation is the following:

– ***Influenza virus* A** RNA is **detected** if the *Ct* value determined in the result grid in the channel for the ROX fluorophore is less than the boundary value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line at the area of typical exponential growth of fluorescence.
– ***Influenza virus* B** RNA is **detected** if the *Ct* value determined in the result grid in the channel for the JOE fluorophore is less than the boundary value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line at the area of typical exponential growth of fluorescence.
– ***Influenza virus* A** RNA and ***Influenza virus* B** RNA are **not detected** in a sample if *Ct* value is not determined (absent) in the channels for ROX and JOE fluorophores, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the value specified in the *Important Product Information Bulletin*.
– The result is **invalid** if the *Ct* value is not determined (absent) in the channel for JOE or ROX fluorophores, whereas *Ct* value in the channel for the FAM fluorophore is not determined (absent) or greater than the specified boundary value. In such cases PCR analysis (beginning with RNA extraction) should be repeated. If the same result is obtained in the second run, re-sampling of material is recommended.
– The result is **equivocal** if the *Ct* value determined in the channel for ROX or JOE fluorophore is greater than the value specified in the *Important Product Information Bulletin*, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the value specified in the *Important Product Information Bulletin.* In such cases PCR analysis should be repeated (beginning with RNA extraction). If the same result is obtained the sample is considered to be positive.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed in 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 the table below).

Table 5

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore		
		FAM	JOE	ROX
C–	RNA extraction	<boundary value	Absent	Absent
NCA	PCR	Absent	Absent	Absent
C+ $_{A/B/STI}$	PCR	<boundary value	<boundary value	<boundary value

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 ROX or JOE fluorophores is greater than the boundary value or absent, amplification and detection should be repeated for all samples in which *Influenza virus* RNA was not detected in the correspondent 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 ROX or JOE fluorophores, PCR analysis (beginning with RNA extraction stage) should be repeated for all samples in which *Influenza virus* RNA was detected in the corresponding channel.

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/B-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

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

NOTE: PCR-mix-1-FL-F *Influenza virus* A/B, 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-FL-F *Influenza virus* A/B is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Pathogen agent	Nucleic acid extraction kit	PCR kit	Sensitivity, GE/ml[4]
Nasopharyngeal and oropharyngeal swabs	*Influenza virus* A	RIBO-sorb, RIBO-prep, NucliSENS easyMAG	variant FRT-100 F	1×10^3
	Influenza virus B	RIBO-sorb, RIBO-prep, NucliSENS easyMAG	variant FRT-100 F	1×10^3

13.2. Specificity

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

The PCR kit allows detection of cDNA fragments of *Influenza virus* A and *Influenza virus* B. The specific activity of the PCR kit was proven in detection of reference strains and isolates of epidemic *Influenza viruses* A/H1N1 and A/H3N2 extracted in the period from 1977 to 2011 in Russian Federation, Ukraine, and Belarus, *Influenza viruses* A extracted from animals (H1N1, H2N2, H2N3, H2N9, H3N2, H3N8, H4N6, H5N1, H5N3, H5N2, H5N3, H6N2, H7N1, H8N4, H9N2, H10N7, H11N6, H12N5, H13N2), *Influenza viruses* B lineages Yamagata and Victoria as well as A/California/07/2009 strain of pandemic *Influenza virus* A/H1N1pdm2009.

The absence of nonspecific reactions of the kit components was shown for cDNA/DNA of other viral (*Human Respiratory-Syncytial virus* "Long" strain, *Human Rhinoviruses* (types 13, 15, 16, 17, 21, 26, and 29), *Herpes viruses*, *Cytomegalovirus*, *Enteroviruses* (types Echo9, Echo30) and bacterial (*Streptococcus* spp., *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Chlamydophila pneumonia*, *Haemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila*) causative agents of acute respiratory disease; normal microflora of human nasal cavity and oropharynx; and human cDNA/DNA as well as analysis of clinical material containing nucleic acids of *Respiratory Syncytial viruses* (types A and B), *Parainfluenza viruses* (types 1-4), *Human Coronavirus* OC43, E229, NL63, HKUI, *Human Adenoviruses* groups B, C, and E, *Human Metapneumovirus,* and *Human Bocavirus.*

The clinical specificity of **AmpliSens® *Influenza virus* A/B-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 the **AmpliSens® *Influenza virus* A/B-FRT** PCR kit for qualitative detection of RNA of *Influenza virus* A and *Influenza virus* B in the biological material 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/B-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
02.06.15 ME	3. Content, Footer	REF R-V36-50-Mod(RG)-CE; REF R-V36-50-Mod(iQ)-CE were changed to REF R-V36-50-Mod-CE
15.08.16 ME	Text	Corrections according to the template
	8.1. RNA extraction	Information about the controls of extraction was added
11.12.18 DV	2. Principle of PCR detection	The information about the enzyme UDG was added
12.02.19 PM	3. Content	The colour of the reagent was specified
15.05.20 EM	Through the text	The text formatting was changed
	Footer	The phrase "Not for use in the Russian Federation" was added
	2. Principle of PCR detection	The table with targets was added
26.10.20 KK	Through the text, Footer	The information about variant FRT REF R-V36-50-Mod-CE was deleted
11.03.21 MA	—	The name, address and contact information for Authorized representative in the European Community was changed

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[4] Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.