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For Professional Use Only

AmpliSens® *Influenza virus* A H5N1-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Influenza virus* A RNA and identifying of H5N1 subtype in the clinical material (nasal and throat swabs or washes; aspirate of trachea; feces; autopsy material) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Influenza virus A H5N1 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Influenza virus* A H5N1 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 the 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 H5N1-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. 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. Wax melts and reaction components mix only at 95 °C. In variant FRT-50 F, “hot-start” is guaranteed by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit is produced in 3 forms:

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit variant FRT (for use with RG) **REF** R-V33(RG)-CE.

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit variant FRT (for use with iQ) **REF** R-V33(iQ)-CE.

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit variant FRT-50 F (for use with SC) **REF** R-V33(SC)-CE.

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit, variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1-FEP/FRT *Influenza virus* A ready-to-use single-dose test tubes *(under wax)*	colorless clear liquid	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 ($C+_A$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.6	3 tubes
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 during the RNA extraction procedure directly to the sample/lysis mixture (see RIBO-sorb **REF** K2-1-Et-50-CE protocol).

Reagents for identifying *Influenza virus* A subtype H5N1:

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1-FEP/FRT *Influenza virus* A H5N1 ready-to-use single-dose test tubes *(under wax)*	colorless clear liquid	0.008	55 tubes of 0.2 ml
Positive Control cDNA *Influenza virus* A H5 ($C+_{H5}$)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *Influenza virus* A N1 ($C+_{N1}$)	colorless clear liquid	0.1	1 tube

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit variant FRT is intended for 55 reactions (including controls).

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit, variant FRT-50 F includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FRT (SC) *Influenza virus* A	colorless clear liquid	0.12	5 tubes
PCR-buffer-Flu	colorless clear liquid	0.28	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Positive Control cDNA *Influenza virus* A ($C+_A$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.6	3 tubes
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 during the RNA extraction procedure directly to the sample/lysis mixture (see “RIBO-sorb” **REF** K2-1-Et-50-CE protocol).

Reagents for identifying *Influenza virus* A H5N1 subtype:

Reagent	***Description***	***Volume (ml)***	***Amount***
PCR-mix-1-FRT (SC) *Influenza virus* A H5N1	colorless clear liquid	0.12	5 tubes
Positive Control cDNA *Influenza virus* A H5 ($C+_{H5}$)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *Influenza virus* A N1 ($C+_{N1}$)	colorless clear liquid	0.1	1 tube

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit variant FRT-50 F is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit.
- 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.
- Rotor-Gene 3000 or Rotor-Gene 6000 Instrument (Corbett Research, Australia); iCycler iQ or iQ5 Instrument (Bio-Rad, USA); SmartCycler II Instrument (Cepheid, USA).
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, 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 RNase-free pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens 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 other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) 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, it must 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® ***Influenza virus*** **A H5N1-FRT** PCR kit is intended for analysis of RNA extracted by using RNA extraction kits from nasal and oropharyngeal swabs or washes, tracheal aspirate, feces, and autopsy material.

6.1. Samples obtained from humans

6.1.1. *Nasal swab samples* are obtained using a probe with a dry cotton swab. Insert the probe gently along the external nasal wall to a depth of 2–3 cm towards the inferior nasal concha. Then move the probe 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, place the working part of the probe with a cotton swab into a sterile disposable tube with 500 µl of sterile saline or phosphate buffer solution. Break off the terminal part of the probe or cut it off to allow tight closing of the tube cup. Close the tube with the solution and the working part of the probe.

6.1.2. *Oropharyngeal swab samples* are obtained using a probe with a dry cotton swab. Obtain swabs by rotating the probe over the surface of tonsils, palatine arches, and posterior wall of pharynx after gargling the oral cavity with water. When material is obtained, place the working part of the probe with the cotton swab into a sterile disposable tube with 500 µl of sterile saline or phosphate buffer solution. Break off the terminal part of the probe or cut it off to allow tight closing of tube cup. Close the tube with the solution and the working part of the

probe.

It is recommended to combine nasal and oropharyngeal swabs in a single tube. For this purpose, place the working parts of both probes into one tube containing 500 µl of transport medium and analyze them as a single sample.

6.1.3. *Nasal wash.* Patient should sit with head tilted backward. Instill 3-5 ml of warm sterile saline solution into each nostril using disposable probe or syringe. Collect the sample from both nostrils in a single sterile tube using funnel. Only an autoclaved funnel should be used.

6.1.4. *Oropharyngeal wash*. It is necessary to rinse the mouth with water before sampling. After that rinse the throat thoroughly with 8–10 ml of saline for 10–15 s. Collect the sample to a sterile tube using a funnel. Only an autoclaved funnel should be used.

6.1.5. *Fecal sample* (1.0 – 3.0 g) should be obtained from a sterile disinfected bedpan or a chamber-pot and transferred to a sterile container with a disposable spatula.

6.1.6. *Autopsy sample* should be immediately placed in a sterile disposable container and frozen otherwise it should be examined within 1 hour from the time of sample collection. Store the samples at minus 68 ºC for 1 year. Only one freeze-thaw cycle of clinical material is allowed.

6.2. Samples obtained from birds

6.2.1. *Droppings* (4.0 – 5.0 g) are collected to a sterile container.

6.2.2. *Cloak, pharyngeal, tracheal swab samples* are obtained with dry sterile cotton swabs. The effective part of the probe is placed in a sterile disposable tube containing 500 µl of respiratory transport medium, sterile saline or phosphate buffer (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM sodium monophosphate, 2mM potassium diphosphate; pH 7.5±0.2. Store phosphate buffer at 2–8 °C for 1 year in a tightly sealed polypropylene tube). Break off the terminal part of the probe or cut it off to allow tight closing of the tube cup. Close tube with solution and working area of the probe.

6.2.3. *Tracheal wash sample* is obtained using a sterile saline.

6.3. Samples obtained from other dead animals

6.3.1. *Visceral organs (fragments of trachea and lungs)* are collected to sterile disposable containers.

6.4. Preparation of clinical material

6.4.1. *Swabs and washes* are used without additional processing.

6.4.2. *Tracheal aspirate.* Mucolysin reagent REF 180-CE is additionally required. Treat samples according to manufacturer's instructions. The prepared solution (50 µl) is used for RNA extraction. The remaining sample can be frozen for further use.

6.4.3. *Autopsy material and visceral organs of animals* should be homogenized using a sterile porcelain mortar and pestle. Then, 10 % suspension in sterile saline or phosphate buffer

should be prepared. Transfer the suspension to a 1.5-ml tube and spin at 10000 rpm for 30 s. Use the supernatant for RNA extraction.

6.4.4. *Human feces*. Prepare fecal suspension from native feces that were not frozen.

Preparation of 10-20% fecal suspension (can be omitted for watery feces).

Take the required number of 1.5-ml tubes. Pipette 0.8 ml of phosphate buffer of sterile saline into each tube. Transfer 0.1 g (0.1 ml) of fecal sample to the tube using a disposable spatula and stir well on vortex to obtain a homogenous suspension.

If the material cannot be studied within 1 day and/or if continuous storage is required, add glycerol (final concentration, 10–15 %) to 10–20 % fecal suspension. Thoroughly homogenize samples with glycerol, incubate for 30–40 min, and then freeze.

Preparation of clarified fecal extract

Vortex the tubes with the prepared suspension (freshly made or frozen with glycerol) or liquid feces, then spin at 10,000 g (12,000 rpm) for 5 min. Use the supernatant for RNA extraction.

6.4.5. *Bird droppings.* Use 4.0–5.0 g of droppings for analysis. Prepare a 10 % suspension in sterile saline, thoroughly resuspend, and decant for 10 min. Transfer the supernatant to an Eppendorf tube and spin it at 12,000 rpm for 5 min. Use the supernatant for RNA extraction.

7. WORKING CONDITIONS

AmpliSens® *Influenza virus* A H5N1-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

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

- RIBO-sorb **REF** K2-1-Et-50-CE.

Extract RNA/DNA according to the manufacturer's instructions.

8.2. Reverse transcription

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

- REVERTA-L, **REF** K3-4-50-CE, which contains RT-G-mix-1.

Carry out the reverse transcription procedure according to the manufacturer's instruction.

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

8.3.1.1. Detection of *Influenza virus* A RNA

Variant FRT

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Influenza virus* A** and wax for amplification of cDNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of 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.**

Variant FRT-50 F

1. Prepare required number of the tubes with **PCR-mix-1-FRT (SC) *Influenza virus* A** (one tube is intended for 11 reactions). Vortex the tube, then centrifuge shortly.
2. For performing N reactions (including 2 controls), mix in a new tube 10*(N+1) µl of **PCR-mix-1-FRT (SC) *Influenza virus* A**, 5.0*(N+1) µl of **PCR-buffer-Flu,** and 0.5*(N+1) µl of **polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer 15 µl of the prepared mixture to each tube for respective PCR instrument.

Steps 3 and 4 are used in both variants.

3. Using tips with aerosol barrier, add **10 µl** of **cDNA samples** obtained at the stage of reverse transcription reaction into the prepared tubes.
4. Carry the control amplification reactions:

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

$C+_A$ - Add **10 µl** of **Positive Control cDNA *Influenza virus* A** to the tube labeled **C_A+** (Positive Control of Amplification).

Step 5 is used only for variant FRT-50 F.

5. Centrifuge the reaction mixture in a Smart Cycler II minicentrifuge.

8.3.1.2. Identifying *Influenza virus* A H5N1 subtype.

cDNA samples with positive results after detection of Influenza virus A RNA are used for identifying of *Influenza virus* A H5N1 subtype.

Variant FRT

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Influenza virus* A H5N1** and wax for amplification of cDNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of 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 H5N1***.*

Variant FRT-50 F

1. Prepare required number of the tubes with **PCR-mix-1-FRT (SC) *Influenza virus* A H5N1** (one tube is intended for 11 reactions). Vortex the tube, then centrifuge shortly.
2. For performing N reactions (including 2 controls) mix in a new tube: 10*(N+1) µl of **PCR-mix-1-FRT (SC) *Influenza virus* A H5N1**, 5.0*(N+1) µl of **PCR-buffer-Flu** and 0.5*(N+1) µl of **polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer 15 µl of the prepared mixture to each tube for respective PCR instrument.

Steps 3 and 4 are applied for both variants.

3. Using tips with aerosol barrier, add **10 µl** of **cDNA samples** obtained at the stage of reverse transcription reaction into the prepared tubes.
4. Carry the control amplification reactions:

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

C+$_{H5}$ - Add **10 µl** of **Positive Control cDNA *Influenza virus* A H5** to the tube labeled **C$_{H5}$+**.

C+$_{N1}$ - Add **10 µl** of **Positive Control cDNA *Influenza virus* A N1** to the tube labeled **C$_{N1}$+**.

Step 5 is applied only for variant FRT-50 F.

5. Centrifuge the reaction mixture in a Smart Cycler II minicentrifuge.

8.3.2. Amplification

8.3.2.1. RG

1. Program the Rotor-Gene according to manufacturer's manual and Guidelines.
2. Create a temperature profile in your Rotor-Gene instrument as follows:
   1. Hold 95 °C – **5 min (variant FRT) or 15 min (variant FRT-50 F)**
   2. Cycling 95 °C – 10 s
      54 °C – 20 s
      72 °C – 10 s
      Cycle repeats– 10 times.
   3. Cycling2 95 °C – 10 s
      54 °C – 20 s – Detection
      72 °C – 10 s
      Cycle repeats – 35 times.
3. Fluorescence is detected on the 2nd stage of Cycling 2 **(54 °C)** in FAM/Green and JOE/Yellow fluorescence channels.
4. Adjust the fluorescence channel sensitivity according to Guidelines.

8.3.2.2. iQ

1. Program the iQ instrument according to manufacturer's manual and Guidelines.
2. Create a temperature profile in your iQ instrument as follows:
   95 °C – **5 min (variant FRT) or 15 min (variant FRT-50 F)**
   10 cycles: 95 °C – 10 s / 54 °C – 25 s /72 °C – 25 s
   35 cycles: 95 °C – 10 s / 54 °C – 25 s (detection) /72 °C – 25 s
3. Fluorescence is detected on the 2nd stage of Cycling 2 **(54 °C)** in FAM and JOE fluorescence channels.
4. Adjust the fluorescence channel sensitivity according to Guidelines.

8.3.2.3. SC

1. Program the Smart Cycler according to manufacturer's manual and Guidelines.
2. Create a temperature profile on your Smart Cycler instrument as follows:

1.	Stage1	Hold	95 °C – 900 s
2.	Stage2	2-Temperature Cycle	95 °C – 15 s
			54 °C – 25 s
			72 °C – 25 s
			Repeat – **42 times**

3. Fluorescence is detected on the 2nd stage of Cycling 2 **(54 °C)** in FAM and Cy3 fluorescence channels.
4. Adjust the fluorescence channel sensitivity according to Guidelines.

9. DATA ANALYSIS

9.1. Detection of *Influenza virus* A RNA

Internal Control is detected in the JOE/Yellow/Cy3 fluorescence channel, *Influenza virus* A cDNA is detected in the FAM/Green fluorescence channel.

See **Guidelines** for data analysis settings for Rotor-Gene 3000 or Rotor-Gene 6000, iCycler iQ or iQ5 and SmartCycler II.

9.2. Identifying of *Influenza virus* A H5N1 subtype

Influenza virus A N1 cDNA is detected on the JOE/Yellow/Cy3 fluorescence channel, *Influenza virus* A H5 cDNA is detected on the FAM /Green fluorescence channel.

See **Guidelines** for data analysis settings for Rotor-Gene 3000 or Rotor-Gene 6000, iCycler iQ or iQ5, and SmartCycler II.

9.3. Results interpretation

The results are interpreted with the software of Rotor-Gene 3000 or Rotor-Gene 6000 Instrument or iCycler iQ or SmartCycler II Instrument by the crossing (or not) of the fluorescence curve with the threshold line.

The results of analysis are accepted as relevant if the results obtained for positive and negative controls of amplification and negative control of extraction are correct.

9.3.1. Detection of *Influenza virus* RNA

Results for controls

Control	Stage for control	Ct channel FAM/Green	Ct channel JOE/Yellow/Cy3	Interpretation
C–	RNA extraction	Neg	Pos (< Y*)	OK
NCA	Amplification	Neg	Neg	OK
$C+_A$	Amplification	Pos (< X*)	Neg	OK

* For X, Y values see Guidelines in case of using Rotor-Gene 3000 or Rotor-Gene 6000 iQ5 or iCycler iQ Instrument or SmartCycler II Instrument.

1. The sample is considered to be positive for *Influenza virus* A if Ct value on FAM/Green channel is less than X.
2. The sample is considered to be negative for *Influenza virus* A if its Ct value is not defined in the results grid on FAM/Green channel and in the results grid in the JOE/Yellow/Cy3 channel

the Ct value does not exceed Y. If the Ct value on FAM/Green channel exceed X, PCR should be repeated. If the same result is achieved or Ct value on FAM/Green channel is less than X, the sample is considered to be positive.

9.3.2. Identifying of *Influenza virus* A H5N1 subtype

Results for controls

Control	Stage for control	Ct channel FAM/Green	Ct channel JOE/Yellow/Cy3	Interpretation
NCA	Amplification	Neg	Neg	OK
C+$_{H5}$	Amplification	Pos (< H*)	Neg	OK
C+$_{N1}$	Amplification	Neg	Pos (< Z*)	OK

* For Z, H values see Guidelines in case of using Rotor-Gene 3000 or Rotor-Gene 6000 Instrument, iCycler iQ or iQ5 Instrument, and in SmartCycler II Instrument.

***Influenza virus* A N1cDNA**

1. The sample is considered to be positive for *Influenza virus* A N1 if Ct value in JOE/Yellow/Cy3 channel is less than Z.
2. The sample is considered to be negative for *Influenza virus* A N1 if its Ct value is not defined in the results grid in JOE/Yellow/Cy3 channel. If Ct value in JOE/Yellow/Cy3 channel exceeds Z, PCR should be repeated. If the same result is achieved or Ct value on JOE/Yellow channel is less than Z, the sample is considered to be positive.

***Influenza virus* A H5 cDNA**

1. The sample is considered to be positive for *Influenza virus* A H5 if Ct value in FAM/Green channel is less than H.
2. The sample is considered to be negative for *Influenza virus* A H5 if its Ct value is not defined in the results grid on FAM/Green channel. If Ct value on FAM/Green channel exceeds H, PCR should be repeated. If the same result is obtained or Ct value in JOE/Yellow channel is less than H, the sample is considered to be positive.

Simultaneous registration of positive signals in the FAM/Green and JOE/Yellow channels indicates that ***Influenza virus* A H5N1** is present in the sample, or several *Influenza virus* subtypes with hemagglutinin 5 and neuraminidase 1 are present simultaneously.

10. TROUBLESHOOTING

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

1. If the signal is detected for C– in the FAM/Green channel (in case of *Influenza virus* A RNA detection) or in any channel (in case of *Influenza virus* A H5N1 subtype detection) and for NCA in any channel, this indicates the contamination of reagents or samples. In this case, the results of analysis of all samples are considered invalid. Repeat analysis of all samples and take measures to detect and eliminate the source of contamination.
2. If no signal is detected for the Positive Controls of amplification, this 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 did not comply 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.

3. If the Ct value in the results grid for IC (the JOE/Yellow/Cy3 channel for PCR-mix-1-FEP/FRT *Influenza virus* A) exceed Y, analysis should be repeated starting from the first step.

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

11. TRANSPORTATION

AmpliSens® *Influenza virus* A H5N1-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 H5N1-FRT** PCR kit (except for Polymerase(TaqF), PCR-mix-1-FRT (SC) *Influenza virus* A and PCR-mix-1-FRT (SC) *Influenza virus* A H5N1) are to be stored at 2–8 °C than not in use. All components of the **AmpliSens® *Influenza virus* A H5N1-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.

Polymerase (TaqF), PCR-mix-1-FRT (SC) *Influenza virus* A, and PCR-mix-1-FRT (SC) *Influenza virus* A H5N1 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, PCR-mix-1-FEP/FRT *Influenza virus* A H5N1, PCR-mix-1-FRT (SC) *Influenza virus* A, and PCR-mix-1-FRT (SC) *Influenza virus* A H5N1 are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Influenza virus* A H5N1-FRT** PCR kit is no less than 5×10^3 copies/ml.

The claimed analytical features of **AmpliSens® *Influenza virus* A H5N1-FRT** PCR kit are guaranteed only when additional reagents kit RIBO-sorb and REVERTA-L (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *Influenza virus* A H5N1-FRT** PCR kit is assured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. The clinical specificity of **AmpliSens® *Influenza virus* A H5N1-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, 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.

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 H5N1-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**$C+_{A}$, $C+_{H5}$, $C+_{N1}$**	Positive control of amplification
RG	For working with Rotor-Gene 3000/6000 (Corbett Research)	**IC**	Internal control
SC	For working with SmartCycler II (Cepheid	**iQ**	For working with iCycler iQ, iQ5 (Bio-Rad)

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
09.12.10	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” 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, PCR-mix-1-FEP/FRT *Influenza virus* A H5N1, PCR-mix-1-FRT (SC) *Influenza virus* A H5N1 and PCR-mix-1-FRT (SC) *Influenza virus* A are kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Amplification	For variant FRT-50 F,15 min duration was added at the 1st step of amplification (95 °C)
	Text	The name AmpliSens® *Influenza virus* A H5/N1-FEP was changed to AmpliSens® *Influenza virus* A H5N1-FEP
27.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”