AmpliSens® *Legionella pneumophila*-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
EC REP	Authorized representative in the European Community	PCE	Positive control of extraction
		IC	Internal control

1. INTENDED USE

AmpliSens® *Legionella pneumophila*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Legionella pneumophila* DNA in the clinical material (tracheal sputum or aspirate, nasopharyngeal and oropharyngeal swabs, bronchial washes or bronchoalveolar lavage, and autopsy material), microorganism cultures, and environmental samples (water, washes from environmental objects, biofilms, and soil) as well as for quantitative detection of *Legionella pneumophila* DNA in water, 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

Legionella pneumophila detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Legionella pneumophila* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that 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® *Legionella pneumophila*-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 using a wax layer. Wax melts and reaction components mix only at 95 ºC.

AmpliSens® *Legionella pneumophila*-FRT PCR kit can be used as:

- a qualitative test for *Legionella pneumophila* DNA detection in the clinical materials. During the test, multiplex real-time PCR of *Legionella pneumophila* mip-gene DNA and protrombin gene DNA is performed. Protrombin gene DNA is used as endogenous internal control. *Legionella pneumophila* mip-gene DNA amplification is detected in the channel for the JOE fluorophore, while the protrombin gene DNA amplification is detected in the channel for the FAM fluorophore. Protrombin gene DNA is a human genome DNA fragment; it should be present in an adequate amount in the DNA sample (no less than 10^3 genome equivalents). Both improper storage conditions and poor DNA extraction process can lead to DNA degradation and loss. So, the endogenous internal control allows not only to control analysis steps but also to estimate the adequacy of sampling and storage.
- a qualitative test for *Legionella pneumophila* DNA detection in environmental samples. In this case the Internal Control STI-338 (IC) is used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. *Legionella pneumophila* mip-gene DNA amplification is detected in the channel for the JOE fluorophore, while the Internal Control STI-338 (IC) DNA amplification is detected in the in the channel for the FAM fluorophore.
- a quantitative test for *Legionella pneumophila* DNA calculation in water. In this case, the Internal Control STI-338 (IC) is used. *Legionella pneumophila* mip-gene DNA amplification is detected in the channel for the JOE fluorophore, while the Internal Control STI-338 (IC) DNA amplification is detected in the in the channel for the FAM fluorophore. To quantify *Legionella pneumophila* and Internal Control DNA copies, quantitative standards are used.

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

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	Internal Control STI-338 (IC) DNA	*Legionella pneumophila* DNA
Target gene	Artificially synthesized sequence (environmental samples) / Protrombin gene DNA (clinical material)	mip-gene DNA fragment

NOTE: Since the degree of water concentration is taken into account in calculations, treat water samples strictly according to this manual.

3. CONTENT

AmpliSens® *Legionella pneumophila*-FRT PCR kit is produced in 1 form:
variant screen-titre-FRT, REF R-B50(RG)-CE.
Variant screen-titre-FRT includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT *Legionella pneumophila* ready-to-use single-dose test tubes (*under wax)*		colorless clear liquid	0.008	70 tubes of 0.2 ml
PCR-mix-2-FL		colorless clear liquid	0.77	1 tube
DNA calibrators	**LS1**	colorless clear liquid	0.06	1 tube
	LS2	colorless clear liquid	0.06	1 tube
	LS3	colorless clear liquid	0.06	1 tube
Positive Control DNA *Legionella pneumophila**		colorless clear liquid	0.5	1 tube
DNA-buffer		colorless clear liquid	0.5	1 tube
Negative Control (C-)		colorless clear liquid	1.6	2 tubes
Internal Control STI-338 (IC)		colorless clear liquid	0.5	1 tube

Variant screen-titre-FRT is intended for 70 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of respiratory swabs.
- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- 0.9 % saline solution or 0.01 M potassium-phosphate buffer (pH 7.0).
- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- DNA extraction kit.
- Pipettes (adjustable).
- Sterile 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)).
- Disposable polypropylene microtubes for PCR (0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves.
- DNA extraction kit.

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 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 specimens and unused reagents in accordance with the 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.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- 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.

Not for use in the Russian Federation

6. SAMPLING AND HANDLING

AmpliSens® *Legionella pneumophila*-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (tracheal sputum or aspirate, nasopharyngeal and oropharyngeal swabs, bronchial washes or bronchoalveolar lavage, and autopsy material), microorganism cultures, and environmental samples (water, washes from environmental objects, biofilms, and soil).

6.1 Sampling

Sampling

Clinical material

- *Tracheal induced sputum* or *aspirate* should be taken to a disposable container after pretreatment.
- *Nasopharyngeal swabs* are obtained using sterile dry flocked swabs with plastic shafts for nasopharyngeal swabs. Gently insert the swab 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, insert the swab into a sterile disposable tube with **500 µl** of **Transport medium for storage and transportation of respiratory swabs** (REF 959-CE, REF 957-CE, REF 958-CE) or sterile saline or potassium-phosphate buffer solution. Break off the end of shaft or cut it off to allow tight closing of the tube cup. Close the tube with the solution and the swab.
- *Oropharyngeal swabs* are obtained using sterile dry rayon swabs with plastic shafts for oropharyngeal swabs. Rotate the swab over the surface of tonsils, palatine arches, and the posterior wall of the pharynx after gargling the oral cavity with water.
  When the material is obtained, insert the swab into a sterile disposable tube with **500 µl** of **Transport medium for storage and transportation of respiratory swabs** (REF 959-CE, REF 957-CE, REF 958-CE) or sterile saline or potassium-phosphate buffer solution. Break off the end of shaft or cut it off to allow tight closing of the tube cup. Close the tube with the solution and the swab.

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

NOTE: Nasopharyngeal and oropharyngeal swabs are used for analysis in case of disease caused by *L. pneumophila* in form of acute respiratory disease (Pontiac fever).

- *Bronchial washes* (bronchoalveolar lavage) in disposable container after pretreatment.
- *Autopsy material* (fragments of affected parts of lungs).

Microorganism cultures suspicious for *Legionella* spp.

Resuspend cultures in 1 ml of saline or potassium-phosphate buffer, then centrifuge at 12,000 rpm for 15 min. The supernatant should be transferred into the disinfectant. Resuspend the sediment in 50 µl of saline solution. Use 50 µl of the suspension for DNA extraction.

Store the above-mentioned material at 2–8 °C for 1 day before the test, at the temperature below minus 16°C for 1 month and at the temperature below minus 68 °C for 1 year. Only one freeze-thawing of the material is allowed.

Environmental samples

- *Water* (wastewater, water from water bodies, and drinking water) (0.5 L) after pretreatment.
- *Wipe samples from environmental objects* are obtained using probe with a swab moistened in a sterile saline solution. The working part of the probe with the swab should be placed in a 1.5-ml tube with 0.5-ml of sterile saline solution. Break off the terminal part of the probe. 50 µl of the solution is used for DNA extraction.
- *Biofilm scraped* from internal surface of water-supply, industrial, and other types of equipment (for example, from trays in air conditioners). Samples of moist biofilms under water or at the water-air interface are obtained with a dry sterile probe (the working part of the probe with a swab is placed in a 1.5-ml tube with 0.5 ml of saline and the other part of probe is broken off and discarded). 50 µl of the sample is used for DNA extraction. Samples of dry biofilms are obtained using a swab saturated in sterile saline. The working part of probe with the swab is placed in a 1.5-ml tube with 0.5 ml of sterile saline solution. Break off the terminal part of the probe. 50 µl of the sample is used for DNA extraction.
- *Soil* (100 g) is collected at sites of presumable bacterial contamination and used after pretreatment.

Store the above-mentioned material at the temperature below 20 °C for 1 week before the test, at the temperature below minus 16°C for 1 month and at the temperature below minus 68 °C for 1 year. Only one freeze-thawing of the material is allowed. Temperature conditions for transportation are not limited.

6.2 Pretreatment

- The sample of *bronchoalveolar lavage* should be mixed by inverting in the initial vessel. Using a tip with aerosol filter, transfer 1.0 ml to a new tube and centrifuge it for 10 min at 10,000 rpm. Decant the supernatant leaving 100 µl of liquid above the pellet. Resuspend the pellet in 100 µl of supernatant and take 50 µl of the suspension for DNA extraction.
- The *sputum* should be treated with **Mucolysin** reagent REF 180-CE according to Mucolysin manual. Use 50 µl of the pretreated sputum for DNA extraction. If it is necessary to repeat the test, freeze the remaining sputum at the temperature below minus 16°C.
- *Autopsy material* is homogenized with a sterile porcelain mortar and pestle, with subsequent preparation of a 10% suspension in sterile saline or potassium-phosphate buffer. Transfer the suspension to a 1.5 ml tube and allow a precipitate to form for 1–3 min. 50 µl of the pretreated supernatant is used for DNA extraction. If it is necessary to repeat the test, store the remaining suspension frozen at the temperature below minus 16°C.
- *Water samples*. 0.5 L of water is preliminary filtered through a paper filter using a glass funnel. After preliminary filtration, water is filtered through a membrane filter with a pore diameter not more than 0.45 µm. After filtration, the membrane filter is cut with sterile scissors (to a disposable Petri dish) and placed with sterile pincers to 1.5-ml tubes with 1 ml of saline solution. The tube is incubated at room temperature for 15–20 min under occasional mixing on vortex to ensure the transition of microflora to the solution. 50 µl of thus obtained solution is used for DNA extraction. The filtrate is to be stored at 2–8 °C for 1 week. It can be frozen at the temperature below minus 16°C in case of longer storage.
- *Soil.* Transfer 0,4–1,0 g (~1.0 ml) of soil into the tubes with tightly close (screw) caps using individual spreader (or disposable paddle). Add 3 ml of saline solution to the each tube, thoroughly mix them and decant for 5 min. The supernatant (50 µl) is to be used for subsequent study.

6.3 Disinfection:

1. **Lysis Solution** from **DNA-sorb-B** kit, REF K1-2-50-CE (if it has been stored at 2–8 °C) should be heated at 60-65 °C until complete crystal dissolution.
2. Add **50 µl** of **Negative Control (C-) reagent** to the pretreated samples (**50 µl**) and mix thoroughly. Then add **300 µl of Lysis Solution**, heat at the temperature (65±1) °C for 15 min.

7. WORKING CONDITIONS

AmpliSens® *Legionella pneumophila*-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA extraction

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

- **DNA-sorb-B,** REF K1-2-50-CE.

The DNA extraction of each test sample (except for clinical material) is carried out in the presence of **Internal Control STI-338 (IC).**

NOTE: Addition of **Internal Control STI-338 (IC)** is not necessary for the samples of clinical material.

Extract DNA according to the manufacturer's protocol taking into account next additions and improvements:

- **Lysis Solution** and **Negative Control (C-) reagent** have been already added to the tubes with test samples (see ***6.3 Disinfection***);
- Using tips with aerosol filter, add **10 µl of Internal Control STI-338 (IC)** to the tubes with prepared environmental samples and microorganism cultures (see **6.3 Disinfection**). Do not add **Internal Control STI-338 (IC)** to the tubes with clinical material (see **6.3 Disinfection**).

NOTE:

- To prepare the Positive control of Extraction add **300 µl** of **Lysis Solution, 50 µl** of **Negative Control (C-) reagent, 10 µl** of **Internal Control STI-338** and **50 µl** of **Positive Control DNA *Legionella pneumophila*** to the tube labeled PCE (Positive Control of Extraction);
- To prepare the Negative Control of Extraction, add **300 µl** of **Lysis Solution, 100 µl** of **Negative Control (C–) reagent** and **10 µl of Internal Control STI-338** to the tube labeled **C–** (Negative control of Extraction).
- After adding **Universal Sorbent, Washing Solution 1, Washing Solution 2** and **TE-buffer for DNA elution** (after incubating at 65°C for 5 min), centrifuge samples at 8,000–10,000 rpm (10,000–13,000 rpm in case of using rotor with the 70 mm radius) each time.

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Legionella pneumophila*** and wax for amplification of DNA 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 *Legionella pneumophila*.**
3. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
4. Carry out the control amplification reactions:

For the qualitative test:

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

LS3 – Add **10 µl** of **DNA calibrator LS3** to the tube labeled **LS3 (Positive Control of Amplification).**

For the quantitative test:

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

LS1 – Add **10 µl** of **DNA calibrator LS1** to the tube labeled **LS1.**

LS2 – Add **10 µl** of **DNA calibrator LS2** to the tube labeled **LS2.**

LS3 – Add **10 µl** of **DNA calibrator LS3** to the tube labeled **LS3.**

Both for qualitative and quantitative tests:

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

PCE – Add **10 µl** of **the sample extracted from the Positive control DNA *Legionella pneumophila* reagent** to the tube labeled PCE (Positive control of Extraction).

8.2.2 Amplification

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

Table 2

Amplification program for rotor-type instruments[1]

Step	Temperature, °C	Time	Cycles
1	95	5 min	1
2	95	10 s	10
	60	20 s	
	72	10 s	
3	95	10 s	35
	56	20 s Fluorescence acquiring	
	72	10 s	

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

2. Adjust the fluorescence channel sensitivity according to the 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.

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

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 IC DNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *Legionella pneumophila* DNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) 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 DNA sample in the corresponding column of the results grid.

9.1 Qualitative test

Principle of interpretation is the following:

– *Legionella pneumophila* DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the specified boundary *Ct* value.
– *Legionella pneumophila* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the specified boundary *Ct* value.

NOTE: Boundary *Ct* values are specified in the Guidelines [2]

The result of the qualitative analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Positive and 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–	DNA extraction	<boundary value	Absent
PCE	DNA extraction	< boundary value	< boundary value
NCA	PCR	Absent	Absent
LS3	PCR	<boundary value	<boundary value

9.2 Quantitative test

For **quantitative test,** use the concentration values for DNA calibrators specified in the *Important Product Information Bulletin*.

Calculation of the quantity of *Legionella pneumophila* DNA copies in 1 ml of a test sample is performed automatically using the software and the specified calibrator values. The obtained result is shown in the respective column of the results grid.

Calculation of concentration values of *Legionella pneumophila* DNA ($C_{DNA\ Lp}$) in 1 l of water is performed according to the following formula or using the software enclosed to the PCR kit:

$$C_{DNA\ Lp}\ (\text{copies/l}) = Q_{DNA\ Lp} / Q_{IC\ STI\text{-}338} * C_{IC\ STI\text{-}338} * 2,$$ **where:**

$C_{DNA\ Lp}$ (copies/l) is the quantity of *Legionella pneumophila* DNA copies in 1 l of water sample,
$Q_{DNA\ Lp}$ (copies/ml) is the calculated quantity of *Legionella pneumophila* DNA copies in 1 ml of a test sample,
$Q_{IC\ STI\text{-}338}$ (copies/ml) is the calculated quantity of Internal Control STI-338 DNA copies in 1 ml of the Internal Control in a test sample,
$C_{IC\ STI\text{-}338}$ (copies/ml) is the number of Internal Control STI-338 DNA copies in 1 ml of Internal Control (specified in the *Important Product Information Bulletin*,
2 - the recalculation coefficient.

NOTE: For quantitation of *Legionella pneumophila* DNA in water samples, each water sample should be tested in two times, starting from the extraction stage. In such case the result is given as an average value of two obtained values.

The results of quantitative analysis are considered reliable only if the obtained value of calculated concentration of the Positive control of Extraction falls in the range specified in *Important Product Information Bulletin.*

10. TROUBLESHOOTING

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

10.1. Qualitative test

1. If the *Ct* value determined for the test sample in the channel for the JOE fluorophore is greater than the specified boundary *Ct* value and the *Ct* value determined in the channels for the FAM fluorophore is less than the specified boundary *Ct* value, the PCR should be repeated. If the same result has been obtained or the *Ct* value determined in the channel for the JOE fluorophore is less than the boundary *Ct* value, the sample is considered positive.
2. If the *Ct* value is not determined (absent) in the channel for 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, the PCR should be repeated. If the same result is obtained in the second run, the PCR analysis (beginning with the DNA extraction stage) should be repeated.
3. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the channels for the JOE fluorophore and for the Negative Control of amplification (NCA) in any of the channels for the FAM and/or JOE fluorophores, this indicates the contamination of reagents or samples. In this case, the results of analysis of all samples are considered invalid. The PCR analysis should be repeated, measures for detecting and elimination of contamination source must be taken.

10.2. Quantitative test

1. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the channels for the JOE fluorophore and for the Negative Control of amplification (NCA) in any of the channels for the FAM and/or JOE fluorophores, this indicates the contamination of reagents or samples. In this case, the results of analysis of all samples are considered invalid. The PCR analysis should be repeated, measures for detecting and elimination of contamination source must be taken.
2. If the concentration value of the *Legionella pneumophila* DNA in the Positive Control of extraction (PCE) does not fall in the range specified in the *Important Product Information Bulletin,* this indicates the errors at extraction or amplification stages. The PCR-analysis must be repeated.
3. If the number of Internal Control STI-338 (IC) DNA copies in 1 ml of test sample is 5 times less than the concentration value of Internal Control STI-338 (IC) DNA specified in the *Important Product Information Bulletin*, this indicates the low efficiency of DNA extraction from the given sample or ineffective cleaning from inhibitors. The PCR analysis (beginning with the DNA extraction stage) should be repeated for the appropriate test sample.

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

11. TRANSPORTATION

AmpliSens® *Legionella pneumophila*-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® *Legionella pneumophila*-FRT** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Legionella pneumophila*-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 *Legionella pneumophila* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® *Legionella pneumophila*-FRT** PCR kit is not less than $1x10^3$ genome equivalents per 1 ml of sample (GE/ml).

NOTE: The claimed analytical features of **AmpliSens® *Legionella pneumophila*-FRT** PCR kit are guaranteed only when additional reagents kit DNA-sorb-B (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") is used.

13.2. Specificity

The analytical specificity of **AmpliSens® *Legionella pneumophila*-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.

Specific activity of the kit was defined by investigation of the following strains from the American Type Culture Collection: *Legionella pneumophila* (serogroups 1-3: *L.pneumophila Philadelphia* 1 (ATCC 33152); *L.pneumophila Togus* 1 (ATCC 33154); *L.pneumophila Bloomington* (ATCC 33155)).

Specificity was proved by the examination of the clinical material from true-negative (healthy) patients and the following cultures of microorganisms: 78 cultures of microorganisms from genus *Bacillus, Citrobacter, Corynebacterium, Enterococcus, Escherichia, Francisella, Klebsiella, Listeria, Proteus, Pseudomonas, Salmonella, Serratia, Shigella, Staphylococcus, Streptococcus, Yersinia* and species of genus of *Legionella* (*L.dumofii, L.longbeachae*). In all cases the negative result has been obtained.

The clinical specificity of **AmpliSens® *Legionella pneumophila*-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® *Legionella pneumophila*-FRT** PCR kit for qualitative detection of *Legionella pneumophila* DNA in the clinical material, microorganism cultures, and environmental samples as well as for quantitative detection of *Legionella pneumophila* DNA in water by 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® *Legionella pneumophila*-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
29.12.10 KM	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 *Legionella pneumophila* is kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
01.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
26.11.11 LA	Text	Information that concentration values of control samples are specified in the Important Product Information Bulletin is added
27.06.17 ME	Through the text	Corrections according to the template and Russian Instruction manual
	6.3 Disinfection	The section was added
	8.1 DNA extraction 9. Data analysis 10. Troubleshooting 13. Specifications	The sections were rewritten
	6. Sampling and handling	In the procedure of nasopharyngeal swabs sampling the probe with cotton swab was changed to flocked swabs with plastic shafts for nasopharyngeal swabs. In the procedure of oropharyngeal swabs sampling the probe with cotton swab was changed to rayon swabs with plastic shafts for oropharyngeal swabs
04.08.20 MA	Through the text	The text formatting was changed. Corrections according to the template
	Footer	The phrase "Not for use in the Russian Federation" was added
	2. Principle of PCR detection	The table with targets was added
	7. Working conditions	The temperature of using the PCR kit from 20 to 28 °C and relative humidity from 15 to 75 % was added

VER	Location of changes	Essence of changes
11.03.21 MA	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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