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For Professional Use Only

AmpliSens® *Legionella pneumophila*-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Legionella pneumophila*-FEP 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, environmental samples (water, washes from environmental objects, biofilms, and soil) using end-point hybridization-fluorescence detection of amplified products.

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 specific *Legionella pneumophila* primers. In Fluorescent End-Point 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. A multi-channel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after the PCR. It allows the detection of the accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *Legionella pneumophila*-FEP PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 ºC.

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

- a qualitative test for *Legionella pneumophila* DNA detection in the clinical materials. During the test, multiplex end-point 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.

3. CONTENT

AmpliSens® *Legionella pneumophila*-FEP PCR kit is produced in 2 forms:

AmpliSens® *Legionella pneumophila*-FEP PCR kit variant FEP (0.5-ml tubes), **REF** B50-50-R0,5-FEP-CE.

AmpliSens® *Legionella pneumophila*-FEP PCR kit variant FEP (0.2-ml tubes), **REF** B50-R0,2-FEP-CE.

AmpliSens® *Legionella pneumophila*-FEP PCR kit 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	55 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
PCR-mix-Background	colorless clear liquid	0.5	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
DNA calibrator LS3	colorless clear liquid	0.06	2 tubes
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

* must be used in the extraction procedure as Positive Control of Extraction.

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

*** add **10 µl** of **Internal Control** during the DNA extraction procedure directly to the sample/lysis mixture (**see DNA-sorb-B**, **REF** K1-2-50-CE protocol).

AmpliSens® *Legionella pneumophila*-FEP PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of respiratory swabs.

- 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.
- Disposable powder-free gloves.
- 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.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia).
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent 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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- 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

AmpliSens® *Legionella pneumophila*-FEP 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

Clinical material

– *Tracheal sputum* or *aspirate* should be taken to a disposable container.

– *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.

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.

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.
- *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*-FEP 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:

- **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).**

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**).

- 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**.

The type of tubes depends on the PCR instrument used for analysis.

8.2.1. Preparing tubes for PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Legionella pneumophila*** and wax for the amplification of DNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer into each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Legionella pneumophila*.**
3. Add above **1** drop of **mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Legionella pneumophila*** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** 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 *Legionella pneumophila***. Add above **1** drop of **mineral oil for PCR.**
5. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
6. Carry out the control amplification reactions:

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).

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. Run the following program on the thermocycler (see Table 1).
2. When the temperature reaches 95 °C (pause mode), insert tubes into the wells of the thermocycler and press the button to continue.

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

Table 1

Amplification program

	Thermocyclers with block temperature adjustment: **MiniCycler, PTC-100 (MJ Research), Uno-2 (Biometra)**			Thermocyclers with active temperature adjustment:					
				GeneAmp PCR System 2400 (Applied Biosystems)			**GeneAmp PCR System 2700 (Applied Biosystems), MaxyGene (Axygen Scientific), Gradient Palm Cycler (Corbett research)**		
Step	Tempe-rapture, °C	Time	Cycles	Tempe-rapture, °C	Time	Cycles	Tempe-rapture, °C	Time	Cycles
0	**95**	pause		**95**	pause		**95**	pause	
1	**95**	2 min	1	**95**	2 min	1	**95**	2 min	1
2	**95**	25 s	10	**95**	10 s	10	**95**	10 s	10
	60	40 s		**60**	20 s		**60**	25 s	
	72	25 s		**72**	10 s		**72**	25 s	
3	**95**	25 s	35	**95**	10 s	35	**95**	10 s	35
	56	40 s		**56**	20 s		**56**	25 s	
	72	25 s		**72**	10 s		**72**	25 s	
4	**72**	1 min	1	**72**	1 min	1	**72**	1 min	1
5	**10**	storage		**10**	storage		**10**	storage	

3. Proceed to fluorescence detection after the amplification program is completed.

9. DATA ANALYSIS

Please read ALA-1/4 Operating Manual before using this kit.

The detection is performed by means of a fluorescence detector by measuring the fluorescence signal intensity in three channels:

– The channel for the FAM fluorophore (FAM channel or analogous, depending on the detector model) is intended for the detection of the signal of the IC DNA amplification product.

– The channel for the JOE fluorophore (HEX channel or analogous, depending on the detector model) is intended for the detection of the signal of the *Legionella pneumophila* DNA amplification product.

Before the detection run, the required settings of the detector software should be adjusted according to the Guidelines [2].

The obtained results are interpreted on the basis of the level of fluorescence signal in the corresponding channels relatively to the background for the clinical and control samples. Interpretation is performed automatically by the software of the instrument used.

The principle of interpretation is the following:

– *Legionella pneumophila* DNA is **detected** if the signal determined in the channel for the JOE fluorophore is greater than the specified threshold value of the positive result;

– *Legionella pneumophila* DNA is **not detected** if the signal determined in the channel

for the JOE fluorophore is less than the specified threshold value of the negative result, whereas the signal determined in the channel for the FAM fluorophore is greater than the specified threshold value.

– The result of the analysis is **invalid** if the signal determined in the channel for the JOE fluorophores is less than the specified threshold value of the negative result and signal determined in the channel for the FAM fluorophore is less than the specified threshold value. In such cases, the PCR analysis of this sample should be repeated starting from the DNA extraction stage.
– The result of analysis is **equivocal**, if the signal of a sample determined in the channel for the JOE fluorophore is greater than the specified threshold value of the negative result but less than the threshold of the positive result (the signal is between the threshold values). In such cases, the PCR of this sample should be repeated. If the negative result is obtained in the second run, the sample is considered equivocal and re-sampling is recommended.

Threshold values are specified in the Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 2)

Table 2

Results for controls

Control	Stage for control	Fluorescent signal in the channel for the fluorophore	
		FAM	JOE
C–	DNA extraction	**>** threshold value	**<** threshold value of negative result
PCE	DNA extraction	**>** threshold value	**>** threshold value of positive result
NCA	PCR	**<** threshold value	**<** threshold value of negative result
LS3	PCR	**>** threshold value	**>** threshold value of positive result

10. TROUBLESHOOTING

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

1. If the signal determined for the Positive Control of amplification (C+) in the FAM channel is less than the threshold value, this may indicate incorrect selection of amplification program and other mistakes of preparing PCR. Repeat the PCR once again.
2. If the signal determined for the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA) in the FAM channel is greater than the threshold value, this indicates contamination of reagents or samples. In this case, the results of analysis

should be considered as invalid. The analysis must be repeated and measures for detecting of contamination source must be taken.

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*-FEP 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*-FEP** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Legionella pneumophila*-FEP** 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.

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*-FEP** PCR kit is not less than $1x10^3$ copies per 1 ml of sample (copies/ml).

The claimed analytical features of **AmpliSens® *Legionella pneumophila*-FEP** PCR kit are guaranteed only when additional reagent 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*-FEP** 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*-FEP** 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, Moscow, 2012.
2. Guidelines to the **AmpliSens® *Legionella pneumophila*-FEP** PCR kit for qualitative detection of *Legionella pneumophila* DNA in the clinical material, microorganism cultures, and environmental samples by polymerase chain reaction (PCR) with end-point 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*-FEP** 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	**LS3**	Positive control of amplification
PCE	Positive control of extraction	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
11.01.11	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
	Footer	Reference numbers were changed from B50-R0,5-FEP-CE; B50-R0,2-FEP-CE to B50-50-R0,5-FEP-CE; B50-50-R0,2-FEP-CE
01.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
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	The sections were rewritten
	9. Data analysis	
	10. Troubleshooting	
	13. Specifications	
	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
06.03.18 PM	Footer, 3.Content	**REF** B50-50-R0,2-FEP-CE was changed to **REF** B50-R0,2-FEP-CE