



For *in Vitro* Diagnostic Use

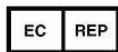
AmpliSens® *Legionella pneumophila*-FEP PCR kit

Instruction Manual



TABLE OF CONTENTS

1. INTENDED USE.....	2
2. PRINCIPLE OF PCR DETECTION.....	2
3. CONTENT.....	2
4. ADDITIONAL REQUIREMENTS.....	3
5. GENERAL PRECAUTIONS.....	3
6. SAMPLING AND HANDLING.....	3
7. PROTOCOL.....	3
8. DATA ANALYSIS.....	6
9. TROUBLESHOOTING.....	7
10. STABILITY AND STORAGE.....	7
11. SPECIFICATIONS.....	7
12. REFERENCES.....	8
13. QUALITY CONTROL.....	8
14. EXPLANATION OF SYMBOLS.....	8



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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 materials (sputum or aspirate from trachea, nasopharyngeal swabs, throat swabs, bronchi scourage or bronchoalveolar lavage, autopsy material), microorganism cultures, environmental samples (water, washes from environmental objects, biofilms scrapes, ground) by using end-point hybridization-fluorescence detection of amplified products.

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 end point PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product in ordinary thermocycler A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run AmpliSens® *Legionella pneumophila*-FEP PCR kit is a qualitative test, which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. The wax melting and reaction mix component occurs only at 95°C.

3. CONTENT.

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

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

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

AmpliSens® *Legionella pneumophila*-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT <i>Legionella pneumophila</i> ready-to-use single-dose	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
LS3	colorless clear liquid	0.06	2 tube
Positive Control DNA <i>Legionella pneumophila</i> (C+)*	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 ST1-338 (IC)***	colorless, clear liquid	0.5	1 tube

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

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

** add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (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.

- DNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), Terzik (DNA-Technology, Russia) or equivalent instrument.
- Fluorometer ALA-1/4 ("Biosan", Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity.
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature not more than –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters 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, 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance 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 contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, seek medical attention
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional; it should begin in the Extraction Area 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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

Clinical material:

1. Sputum or aspirate from trachea in disposable container after preliminary preparation.

2. Nasopharyngeal and throat swabs (in transport medium for storage and transportation of respiratory swabs). These samples do not need the preliminary preparation.
3. Bronchi scourage (bronchoalveolar lavage) in disposable container after preliminary preparation.
4. Autopsy material (fragments of affected lungs part) after preliminary preparation.



It is recommended to combine nasopharyngeal and throat swabs. For this working ends of probes are placed in one tube with 500 µl of medium for storage and transportation of respiratory swabs and studied as one sample.

Nasopharyngeal swabs are obtained by probe with dry cotton swab. Insert probe gently along the external nasal wall on 2–3 cm till the inferior nasal concha. Then move the probe slightly lower, insert in the inferior nasal meatus under the inferior nasal concha, rotate and remove along the external nasal wall.

When material is obtained insert the working area of the probe with cotton swab to sterile disposable tube with 500 µl of sterile saline or phosphate buffer solution. Broke off the terminal part of the probe or cut it off to allow dense closing of tube cup. Close tube with solution and working area of the probe.

Throat swabs are obtained by probe with dry cotton swab. Obtain smears by rotating the probe at the surface of tonsils, palatine arches, posterior wall of pharynx after gargling of oral cavity with water.

When material is obtained insert the working area of the probe with cotton swab to sterile disposable tube with 500 µl of sterile saline or phosphate buffer solution. Broke off the terminal part of the probe or cut it off to allow dense closing of tube cup. Close tube with solution and working area of the probe.

The sample of bronchoalveolar lavage should be mixed in original cap. Using tip with aerosol barrier transfer 1.5 ml to the separate tube and centrifuge for 10 min at 10 000 rpm. Decant the liquid, leaving 100 µl above the pellet. Resuspend the pellet in 100 µl of supernatant. 50 µl of suspension is used for DNA extraction.

The sputum must be treated with «Mucolysin» reagent REF 180, according to «Mucolysin» manual. 50 µl of pretreated sputum is used for DNA extraction. In case of test repeat freeze the rest of the sputum.

Section material is homogenized with sterile porcelain mortar and pestle, with subsequent preparation of 10% suspension on sterile saline or phosphate buffer. Transfer suspension to 1.5 ml tube and let the pellet settle down within 1-3 min. 50 µl of pretreated supernatant is used for DNA extraction. In case of test repeat freeze the rest of the suspension at no more than minus 16°C.

Nasopharyngeal and throat swabs are used for analysis in case of legionellosis in acute respiratory disease form (Pontiac fever). In case of pneumonia *Legionella pneumophila* DNA can be detected in throat swabs, urine and blood plasma, but in insignificant percent of cases. So analysis of this clinical material can be conducted if sampling of sputum, aspirate or bronchoalveolar lavage is impossible. Negative result of this analysis is not final. If *Legionella pneumophila* DNA is detected in throat swabs, urine and blood plasma, the positive result is final.

Microorganism cultures, suspected of *Legionella* spp

Resuspend cultures in 0.5 ml of saline solution or phosphate buffer. Use 50 µl of suspension for DNA extraction.

All this material can be stored for 1 day at 2 – 8°C, 1 month at no more than minus 16°C and 1 year at no more than minus 68°C.



Only one freeze-thaw cycle is allowed.

Environmental samples

- Water (wastewater, from water reservoir, drinking water) of 0.5 L after preliminary preparation. 0.5 L of water is preliminary filtered through paper filter with glass funnel. After preliminary filtration water is filtered through membrane filter with pore diameter not more than 0.45 µm. After filtration membrane filter is chopped by sterile scissors (to disposable Petri dish) and placed by sterile pincers to 1.5 ml tubes with 1 ml of saline solution. The tube is incubated at room temperature during 15-20 min, periodically mixing on vortex for ensuring of microflora transition in solution. 50 µl of solution is used for DNA extraction.
- Washes from environmental objects are obtained by probe with cotton swab, saturated in sterile saline solution. Working end of probe with swab is placed in tube with 1.5 ml of saline solution, another part of probe is broken off and moved away. 50 µl of sample is used for DNA extraction.
- Biofilms scrapes from internal surface of water supply, industrial and other equipment (for example, from tray inside air-conditioners). Scrapes of moist biofilms under water or on the water-air interface are obtained by dry sterile probe (working end of probe with swab is placed in 1.5 ml tube with 0.5 ml of saline solution, another part of probe is broken off and moved away). 50 µl of sample is used for DNA extraction. Scrapes of dried biofilms are obtained by swab, saturated in sterile saline solution. Working end of probe with swab is placed in 1.5 ml tube with 0.5 ml of saline solution, another part of probe is broken off and moved away). 50 µl of sample is used for DNA extraction.
- Ground (100 g) is obtained in places of supposed seeding and used after preliminary preparation. Transfer the ground (0.4-1.0 g) to the tubes of 5 ml with tightly closable lid. Add 3 ml of saline solution in each tube, mix careful and decant 5 min. Supernatant (50 µl) is used for DNA extraction.

All this material can be stored for 1 week at room temperature. Further storage is allowed at no more than minus 16°C. Transportation of these samples is allowed at any temperature.



Only one freeze-thaw cycle is allowed.

7. PROTOCOL.

7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kit:

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



Carry the DNA isolation according to the manufacturer's instructions.



Add 10 µl of Internal Control STI-338 into tubes with environmental samples.



For Positive Control (PC) add 50 µl of Positive Control DNA *Legionella pneumophila* and 50 µl of Negative Control into the tube.

The sample volume is 50 µl. Add 50 µl of Negative Control into each tube.

7.2. Preparing the PCR.

Total reaction volume - **25 µl**, volume of DNA sample - **10 µl**.

7.2.1 Preparing tubes for PCR.

- 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.

- 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***.
- Add above **1** drop of **mineral oil for PCR** (about **25 µl**).
- 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**.
- Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage.
- Carry 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 **LS3** to the tube labeled LS3 (Positive Control of Amplification).

7.2.2 Amplification.

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

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before placing them in the thermocycler.

Table 1

Programming thermocyclers at DNA amplification of *Legionella pneumophila*

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

8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to the manufacturer's manual and Appendix 1.

8.2. Results interpretation

1. When the analysis is complete the results are automatically shown in the table as follows:

pos – positive result; **neg** – negative result;

eq – equivocal result (signal is in grey zone);

nd – invalid result (specific signal and IC signal are absent in the sample).

2. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2).

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (IC)	HEX channel (samples)	
C-	DNA isolation	+	<i>Legionella</i> - neg	OK
PCE	DNA isolation	+	<i>Legionella</i> - pos	OK
NCA	Amplification	-	<i>Legionella</i> - nd	OK
LS3	Amplification	+	<i>Legionella</i> – pos	OK

9. TROUBLESHOOTING.

If analysis results are not obtained as per the following examples:

- Preparing the PCR and detection should be repeated for samples with result **nd** (except for NCA). In the case of analogous result, it is necessary to repeat the sample analysis, beginning with extraction stage. For the NCA sample the result **nd** is normal.
- Preparing the PCR and detection should be repeated for samples with result **eq**. In the case of analogous result, the samples are considered to be positive.
- No positive signal in positive control of PCR (LS3) can indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components not complying with manufacturer instruction, or reagent kit has expired. Check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- Positive signal in negative controls (C-, NCA) indicates the reagent or sample contamination. In this case results of the analysis are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® Legionella pneumophila-FEP** PCR kit are to be stored between 2 °C and 8 °C, when not in use. They also must be stable until the expiry date stated on the label.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Legionella pneumophila-FEP** PCR kit is no less than 1×10^3 copies per 1 ml of sample (copies/ml).



The claimed analytical features of **AmpliSens® Legionella pneumophila-FEP** PCR kit are guaranteed only when additional reagents kit DNA-sorb-B (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) is used.

11.2. Specificity.

Specificity of **AmpliSens® Legionella pneumophila-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® Legionella pneumophila-FEP** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES.

- Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science Central Research Institute of 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.

14. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ iCycler



Negative control