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For Professional Use Only

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection and quantitation of DNA of *Listeria monocytogenes* in the biological material (DNA samples taken from peripheral and umbilical cord blood, cerebro-spinal fluid, node aspirates, naso-pharyngeal swabs, the discharge of the eye conjunctiva, amniotic fluid, placenta, the swabs of epithelial cells taken from vagina side parietes, urine, breast milk, meconium, faeces, autopsy material, primary aliment fortification medium) by using real-time 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

Listeria monocytogenes detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Listeria monocytogenes* 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 fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

The DNA extraction is carried out with the internal control sample (IC STI-87) which helps control the test procedure for each sample. During the extraction of DNA from the biological (peripheral and umbilical cord blood, cerebro-spinal fluid, node aspirates, naso-pharyngeal swabs, the discharge of the eye conjunctiva, amniotic fluid, placenta, the scrapes of epithelial cells taken from vagina side parietes, urine, breast milk, meconium, faeces) and autopsy material the amplification of the human genome DNA takes place (endogenous internal control). Endogenous internal control (IC Glob) gives the opportunity not only to control the stages of PCR analysis (the DNA extraction and carrying out of PCR), but to evaluate the adequacy of the sampling and the storage of the material as well. Then the amplification of *L.monocytogenes* DNA is carried out using the specific for the DNA primers and Taq-polymerase enzyme. The reaction mix contains fluorescently labeled oligonucleotide probes which hybridize with the complementary area of the amplified DNA-target, as the result the fluorescence

intensity grows. The fluorescence signal detection is carried out using the thermo cycler with the system of fluorescence signal detection in real-time mode.

3. CONTENT

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT PCR kit is produced in one form:

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT PCR kit variant FRT-100 F, **REF** R-B14-100-FT(RG,iQ)-CE

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT PCR kit variant FRT-100 F includes:

Reagent		*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *Listeria monocytogenes*		colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT		colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)		colorless clear liquid	0.03	2 tubes
DNA calibrators	**C1 *LIM***	colorless clear liquid	0.1	2 tubes
	C2 *LIM*	colorless clear liquid	0.1	2 tubes
TE-buffer		colorless clear liquid	0.5	1 tube
Negative Control (C–)*		colorless clear liquid	1.2	1 tube
Positive Control DNA *Listeria monocytogenes* and human DNA**		colorless clear liquid	0.1	2 tubes
Internal Control STI-87 (IC)***		colorless clear liquid	0.6	2 tubes

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

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

*** add 10 µl of Internal Control STI-87-rec during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, **REF** K1-9-Et-50-CE protocols).

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free 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); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
    a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
    b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used;
- Refrigerator at 2 to 8 °C.
- Deep-freezer at 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 barriers and use 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 protective gloves and laboratory cloths, and protect eye 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 compliance with appropriate biosafety practices.
- Clean and disinfect all specimens or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid specimens and reagents contact with the skin, eyes, and mucous membranes. If 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, 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

Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT PCR kit is intended for analysis of DNA extracted with DNA extraction kits from the biological material (DNA samples taken from peripheral and umbilical cord blood, cerebro-spinal fluid, node aspirates, naso-pharyngeal swabs, the discharge of the eye conjunctiva, amniotic fluid, placenta, the scrapes of epithelial cells taken from vagina side parietes, urine, breast milk, meconium, faeces, autopsy material, primary aliment fortification medium).

Sampling

The material for the analysis includes DNA samples extracted from:

- peripheral and umbilical cord blood;
- cerebro-spinal fluid;
- node aspirates;
- naso-pharyngeal swabs;
- the discharge of the eye conjunctiva;
- amniotic fluid;
- placenta;
- the scrapes of epithelial cells taken from vagina side parietes;
- urine;
- breast milk;
- meconium;
- faeces;
- autopsy material;
- primary aliment fortification medium;

- the medium for primary fortification of environment objects (concentrated (eluated) water samples (discharged water, drinking water taken from land-based bodies of water, etc).

Pretreatment

- The pretreatment of peripheral and umbilical cord blood is carried out in the following way: add 1.0 ml of hemolytic and 0.25 ml of blood into 1.5-ml “Eppendorf”-type tubes using separate tips. Gently mix the content of the tube using a vortex and leave it for 10 minutes stirring at times. Centrifuge the tubes using a microcentrifuge at 6,000 g (8,000 rpm) during 2 min. The supernatant liquid is to be taken with a vacuum aspirator leaving the deposit untouched. After washing the cells deposit (the leftovers of destroyed erythrocytes) should be white (only the pinkish coat above the deposit is acceptable). One can repeat the washing using hemolytic if necessary. The deposit of leucocytes is to be immediately lysed (in case of using “RIBO-prep” extraction kit add 300 µl of lysis solution and proceed to the extraction of DNA in accordance with the Instruction Manual to “RIBO-prep” kit not adding the lysis solution once again) or long-lastingly stored frozen at minus 68 °C and lower.
- Amniotic fluid is to be extracted into a sterile “Eppendorf”-type tube during the amniocentesis in accordance with the standard technique. It is required to carry out the pretreatment of the test material. Resuspend the amniotic fluid thoroughly. Take 1 ml of material with an adjustable pipette using a filter tip and pour it into a sterile “Eppendorf”-type tube for further centrifugation at 8,000-9,000 g (12,000–13,000 rpm in a centrifuge with 12 wells) during 10 min. After the process of centrifugation carefully take the supernatant fluid with a filter tip leaving 200 µl of fluid above the deposit. Then resuspend the material on the vortex. It is acceptable to store the amniotic fluid and pretreated material within 24 hours at 2-8 °C, within 1 month at minus 16 °C and lower. Long-term storage is allowed at minus 68 °C.
- Breast milk is to be pretreated before testing. Stir the breast milk sample by pipetting. Take 1 ml of material with an adjustable pipette using a filter tip and pour it into a sterile “Eppendorf”-type tube for further centrifugation at 8,000-9,000 g (12,000–13,000 rpm in a centrifuge with 12 wells) during 5 min. Carefully take the supernatant leaving 100 µl of supernatant fluid, then resuspend the material on the vortex. It is acceptable to store the breast milk deposit within 24 hours at 2-8 °C, within 1 month at minus 16 °C and lower. Long-term storage is allowed at minus 68 °C.

- Concentrated (eluated) water samples are used for the extraction of *L.monocytogenes* DNA without any pretreatment. If there are any visible foreign substances or visible tinction in the test samples, the samples are to be stirred thoroughly on the vortex, then one should carry out the centrifugation during 1 min at 10,000 g at room temperature. The supernatant fluid is used for the DNA extraction. The material is to be stored within 24 hours at 2–8 °C, within 1 month at minus 16 °C and lower. Long-term storage is allowed at minus 68 °C.

7. WORKING CONDITIONS

AmpliSens® *Listeria monocytogenes*-screen-titre-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 kits:

- RIBO-prep, **REF** K2-9-Et-50-CE

Extract DNA according to the manufacturer's protocol.

DNA is extracted from each clinical sample in the presence of internal control sample, **Internal Control STI-87 (IC)** (**10 µl** of **Internal Control STI-87 (IC)** is added to each sample).
Transfer **100 µl** of **Negative Control** to the tube labeled C–.
Transfer **90 µl** of **Negative Control** and **10 µl** of **Positive Control DNA *Listeria monocytogenes* and human DNA** to the tube labeled PCE.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

The choice of amplification tubes depends on the thermo-cycler with the system of real-time detection.

Disposable filter tips are used for adding reagents, DNA samples and control samples into the tubes.

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

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. Pour all the content of the tube with **polymerase (TaqF)** into the tube with **PCR-mix-2-FRT** and stir it carefully on the vortex not allowing foaming. Label the tube indicating the date of the preparation of the mix.

The mixture is to be stored at 2–8 °C within 3 months and used as and when necessary.

If the mix can't be used within three months it is necessary to prepare the mix for less number of reactions – for example mix 150 µl of **PCR-mix-2-FRT** and 15 µl of **polymerase (TaqF)** (such mix is intended for 30 reactions).

2. Prepare the reaction mix. Account must be taken of the following: for testing any number of DNA samples in the qualitative format it is necessary to set two control points of the amplification stage: the positive control (**DNA calibrator C2 *LIM***) and the negative control of amplification (**TE-buffer**). In the quantitative format it is necessary to set five control points of the amplification stage: *two* reruns of two calibrators (**C1 *LIM*** and **C2 *LIM***) and the negative control of amplification (**TE-buffer**). It is also necessary to take the reagents in ample amount reckoning upon one extra reaction.
3. In a separate tube mix **PCR-mix-1-FL *Listeria monocytogenes*** with the prepared mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).** The calculation is performed basing on the assumption that each run of PCR is to be carried out with:
   - **10 µl** of **PCR-mix-1-FL *Listeria monocytogenes;***
   - **5 µl** of the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).**

One can calculate the needed number of reactions according to the scheme given in the Table 1.

Table 1

Scheme of reaction mixture preparation

The total reaction volume is 25 µl, including the volume of DNA sample – 10 µl			
Reagent volume per one reaction, µl		**10.0**	**5.0**
Number of clinical samples		**PCR-mix-1-FL *Listeria monocytogenes*[1]**	**The mixture of PCR-mix-2-FRT and polymerase (TaqF)**
For quantitative detection	**For qualitative detection**		
1	**4**	**70**	**35**
2	**5**	**80**	**40**
3	**6**	**90**	**45**
4	**7**	**100**	**50**
5	**8**	**110**	**55**
6	**9**	**120**	**60**
7	**10**	**130**	**65**
8	**11**	**140**	**70**
9	**12**	**150**	**75**
10	**13**	**160**	**80**

[1] The values are given with due regard to the ample number of reactions (reckoning upon one extra reaction) and to the necessity to set five control points: two reruns of two calibrators (**C1 *LIM*** and **C2 *LIM***) and the negative control of PCR (**TE-buffer**) for qualitative detection of *L.monocytogenes* DNA and two control points (positive and negative control samples) for qualitative detection of *L.monocytogenes* DNA.

11	**14**	**170**	**85**
12	**15**	**180**	**90**
13	**16**	**190**	**95**
14	**17**	**200**	**100**
15	**18**	**210**	**105**
16	**19**	**220**	**110**
17	**20**	**230**	**115**
18	**21**	**240**	**120**
19	**22**	**250**	**125**
20	**23**	**260**	**130**
21	**24**	**270**	**135**
22	**25**	**280**	**140**
23	**26**	**290**	**145**
24	**27**	**300**	**150**
25	**28**	**310**	**155**
30	**33**	**360**	**180**

4. Take the needed number tubes for the amplification of the test samples and control samples of DNA. Select the type of the tube depending on the instrument used.
5. Drip **15 µl** of the reaction mix into the tubes.
6. Add **10 µl** of DNA samples extracted from the clinical or control samples into the tubes with the reaction mix.
7. Carry out control reactions:

for qualitative analysis:

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

C+ - Add **10 µl** of DNA calibrator **C2 *LIM*** to the tube labeled **Positive Control** of PCR (**C+**)

for quantitative analysis:

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

DNA calibrators - Add **10 µl** of DNA calibrator **C1 *LIM*** into two tubes and **10 µl** of DNA calibrator **C2 *LIM*** into two other tubes

8.2.2. Amplification

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

Table 2

Amplification program

	***Rotor-type instruments*[2]**			***Plate-type instruments*[3]**		
Cycle	***Temperature, °C***	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s Fluorescence acquiring		**60**	30 s Fluorescence acquiring	
	72	15 s		**72**	15 s	

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

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin* and 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.

9. DATA ANALYSIS

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

- The signal of the Internal Control STI-87 (IC) amplification product is detected in the channel for the FAM fluorophore.
- The signal of the β-globin gene (IC Glob) amplification product is detected in the channel for the ROX fluorophore.
- The signal of the *L.monocytogenes* 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 a 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.

The principle of interpretation is the following:

If DNA is extracted from peripheral and umbilical cord blood, placenta, the scrapes of

[2] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene (QIAGEN, Germany).
[3] For example, iCycler iQ, iQ5 (Bio-Rad, USA).

epithelial cells taken from vagina side parietes, node aspirates, and autopsy material:

- ***L.monocytogenes*** DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***L.monocytogenes*** DNA is **not detected** in a sample if *Ct* value is not determined (absent) in the channels for JOE fluorophores, whereas the *Ct* value determined in the channel for the ROX fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin*.
- The result is **invalid** if *Ct* value is not determined (absent) in the channel for JOE fluorophores, whereas the *Ct* value in the channel for the ROX fluorophore is not determined (absent) or greater than the specified boundary Ct value. In such cases, PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.
- The result is **equivocal** if the *Ct* value determined in the channel for JOE fluorophore is greater than the boundary Ct value specified in the *Important Product Information Bulletin*, whereas the *Ct* value determined in the channel for the ROX fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin.* In such cases, PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained, the sample is considered positive.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for the control samples of the DNA extraction and PCR are correct (see Table 3):

Table 3

Results for controls If DNA is extracted from peripheral and umbilical cord blood, placenta, the scrapes of epithelial cells taken from vagina side parietes, node aspirates, and autopsy material

Control	Stage for control	*Ct* value in the channel for fluorophore			
		JOE		ROX	
		Qualitative format	Quantitative format	Qualitative format	Quantitative format
C–	DNA extraction	Absent	Absent	Absent	Absent
PCE	DNA	**<**boundary value	within boundary	**<**boundary value	**<**boundary value

	extraction		value		
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	**<**boundary value	-	**<**boundary value	-
C1 *LIM* C2 *LIM*	PCR	-	*Ct* value and calculated concentration are detected	-	*Ct* value and calculated concentration are detected

If DNA is extracted from amniotic fluid, breast milk, cerebro-spinal fluid, naso-pharyngeal swabs, the discharge of the eye conjunctiva, urine, meconium, faeces, primary aliment fortification medium, the medium for primary fortification of environment objects:

- ***L.monocytogenes*** DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***L.monocytogenes*** DNA is **not detected** in a sample if *Ct* value is not determined (absent) in the channels for JOE fluorophores, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin*.
- The result is **invalid** if *Ct* value is not determined (absent) in the channel for JOE fluorophores, 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, PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.
- The result is **equivocal** if the *Ct* value determined in the channel for JOE fluorophore is greater than the boundary Ct value specified in the *Important Product Information Bulletin*, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin.* In such cases, PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained, the sample is considered positive.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed in the PCR kit. See also Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for the

control samples of the DNA extraction and PCR are correct (see Table 4):

Table 4

Results for controls If DNA is extracted from amniotic fluid, breast milk, cerebro-spinal fluid, naso-pharyngeal swabs, the discharge of the eye conjunctiva, urine, meconium, faeces, primary aliment fortification medium, the medium for primary fortification of environment objects

Control	Stage for control	*Ct* value in the channel for fluorophore			
		JOE		FAM	
		Qualitative format	Quantitative format	Qualitative format	Quantitative format
C–	DNA extraction	Absent	Absent	<boundary value	<boundary value
PCE	DNA extraction	<boundary value	within boundary value	<boundary value	<boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	<boundary value	-	<boundary value	-
C1 *LIM* C2 *LIM*	PCR	-	*Ct* value and calculated concentration are detected	-	*Ct* value and calculated concentration are detected

For the quantitative test one is to carry out the calculation of the DNA *L.monocytogenes* concentration per 1 ml using the following formula:

The calculation concentration of the DNA L.monocytogenes x coefficient A×100 = copies/ml

$$Coefficient\ A = \frac{100}{extraction\ volume,\ \mu l}$$

10. TROUBLESHOOTING

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

1. If the *Ct* value for the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in the channel for **JOE** fluorophore determined it is necessary to repeat the PCR once again for all the samples in which the presence of *L.monocytogenes* DNA was detected beginning with the extraction of DNA. It is also necessary to take measures for the detection of the contamination source.
2. The absence of the positive signal in calibrators may show that the amplification program was chosen incorrectly and other errors that took place at the PCR stage. In this case it is necessary to carry out the PCR for all the samples once again.
3. If the result for the test sample is positive there is no area of the typical exponential growth in the fluorescence plot (the plot represents an approximately straight line). It may indicate that the set level of the threshold line or calculation parameters of the basal line were incorrect. This result is not to be determined as positive. If it was

obtained at the correct level of the threshold line it is necessary to carry out the PCR for this sample once again.

11. TRANSPORTATION

AmpliSens® *Listeria monocytogenes*-screen-titre-FRT PCR kit should be transported at 2-8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

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

PCR-mix-1-FL *Listeria monocytogenes*, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored from minus 24 °C to minus 16 °C

PCR-mix-1-FL *Listeria monocytogenes* is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Nucleic acid extraction kit	Analytical sensitivity, copies/ml	Linear range, copies/ml
Peripheral and umbilical cord blood; cerebro-spinal fluid; node aspirates; naso-pharyngeal swabs; the discharge of the eye conjunctiva; amniotic fluid; placenta; the scrapes of epithelial cells taken from vagina side parietes; urine; breast milk; meconium; faeces; autopsy material; primary aliment fortification medium; the medium for primary fortification of environment objects (concentrated (eluated) water samples (discharged water, drinking water taken from land-based bodies of water, etc)	RIBO-prep	500	$8x10^2 – 1x10^7$

13.2. Specificity

The analytical specificity of **AmpliSens® *Listeria monocytogenes*-screen-titre-FRT**

PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The PCR kit detects *L.monocytogenes* DNA. The specific activity of the PCR kit is proved by testing different strains of *L.monocytogenes.* The absence of the kit components' activity is shown in reference of DNA of other causative agents such as: *Candida albicans*, *Chlamydophila pneumonia*, *Cryptococcus neoformans*, *Cytomegalovirus hominis*, *Epstein–Barr virus* (*EBV*), *Escherichia coli*, *Haemophilus haemolyticus*, *H.influenzae*, *H.parainfluenzae*, *Hepatitis A virus* (*HAV*), *Hepatitis B virus* (*HBV*), *Hepatitis C virus* (*HCV*), *Hepatitis D virus* (*HDV*), *Herpes simplex virus I* (*HSV I*), *Herpes simplex virus II* (*HSV II*), *Human Herpes virus VI* (*HHV6*), *Human Herpes virus VII, VIII* (*HHV7*), *Human adenovirus B*, *C*, *E*, *F*; *Human immunodeficiency virus* (*HIV*), *Human papillomavirus* 6, 11, 16, 18, 33, 35 (*HPV* 6, 11, 16, 18, 33, 35), *Klebsiella oxytoca*, *K.pneumonia*, *Measles virus*, *Moraxella catarrhalis*, *Mumps virus*, *Mycobacterium tuberculosis*, *Mycoplasma pneumonia*, *Neisseria cinereae*, *N.elongata*, *N.flavescens*, *N.gonorrhoeae*, *N.meningitidis*, *N.mucosa*, *N.sicca*, *N.subflava*, *Proteus mirabilis*, *P.vulgaris*, *Pseudomonas aeruginosa*, *Rubella virus*, *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *S.milleri*, *S.mitis*, *S.mutans*, *S.oralis, S.pneumoniae*, *S.pyogenes*, *S.salivarius*, *S.sanguis*, *S.suis*, *S.viridans*, *Toxoplasma gondii*, *Varicella–Zoster virus* and human DNA.

The clinical specificity of **AmpliSens® *Listeria monocytogenes*-screen-titre-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, Moscow, 2008.
2. Guidelines to the **AmpliSens® *Listeria monocytogenes*-screen-titre-FRT** PCR kit for detection and quantitation of Listeria monocytogenes DNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Listeria monocytogenes*-screen-titre-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
RUO	Research Use Only		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
	Caution	**IC**	Internal control
		PCE	Positive control of extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
10.12.13 ChA	3. Content, Text	The name of the reagent Polymerase (Taq F) was changed to Polymerase (TaqF)
		The name of the reagent Positive Control DNA *Listeria monocytogenes* (C+$_{L.\ monocytogenes}$) and human DNA was changed to Positive Control DNA *Listeria monocytogenes* and human DNA
		The name of the reagent Internal Control STI-87 (IC STI-87) was changed to Internal Control STI-87 (IC)
		The abbreviation of Positive control of extraction C+ was changed to PCE
	8.1. DNA extraction	The chapter was corrected
	8.2.1. Preparing tubes for PCR	Table 1 “Scheme of reaction mixture preparation” was added
		Description of Control reactions carrying out for qualitative and quantitative analysis was corrected in accordance with the pattern
	14. References	The name of guidelines was corrected