For Professional Use Only

eSens Listeria monocytogenenes QT PCR kit

REF ES3402B

Instructions for Use

1 INTENDED USE

eSens Listeria monocytogenes QT PCR kit is an *in vitro* nucleic acid amplification test for detection and quantitation of DNA of *L. monocytogenes* in the biological material (whole blood, umbilical cord blood, cerebro-spinal fluid (CSF), node aspirates, swabs from the respiratory tract, swabs from the eye conjunctiva, amniotic fluid, placenta, swabs from vagina mucous membrane, urine, breast milk, meconium, faeces, autopsy material, environmental samples (concentrated water samples (wastewater, potable water, surface water)), liquid medium for primary enrichment of food product) 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

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 the real-time 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. 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.

eSens Listeria monocytogenes QT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87 (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

The quantitative analysis of *Listeria monocytogenes* DNA is based on the linear dependence between the initial concentration of DNA target in a test sample and the cycle threshold (*Ct*) (the cycle of beginning of fluorescence signal exponential growth). For the quantitative analysis amplification of DNA from the test samples is carried out simultaneously with DNA-calibrators (samples with the known concentration of the DNA target). Based on the amplification results of DNA-calibrators a calibration line is plotted and it is used for the estimation of concentration of the DNA target in the test samples.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glicosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

At the amplification stage 3 reactions are carried out in one tube simultaneously: amplification of DNA fragments of *L. monocytogenes* and Internal Control STI-87 (IC) as well as amplification of human DNA fragment (IC Glob). The results of amplification of *L. monocytogenes*, Internal Control STI-87 (IC) and IC Glob DNA are registered in 3 different fluorescence channels.

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

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**
DNA-target	Internal Control STI-87 (IC) DNA	*L. monocytogenes* DNA	DNA fragment of β-globin gene (IC Glob)
Target gene	Internal Control STI-87 (IC) DNA	listeriolysin O (*hly*) gene	β-globin gene

3 CONTENT

eSens Listeria monocytogenes QT PCR kit (ES3402B) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FL ***Listeria monocytogenes***	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-H	colorless clear liquid	0.3	1 tube
C1 *LIM*	colorless clear liquid	0.2	1 tube
C2 *LIM*	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Internal Control STI-87 (IC)*	colorless clear liquid	0.6	1 tube
Negative Control (C–)**	colorless clear liquid	1.2	1 tube
Positive Control *Listeria monocytogenes****	colorless clear liquid	0.1	1 tube

* add **10 µl** of **Internal Control STI-87 (IC)** during the DNA extraction procedure directly to the sample/lysis mixture.

** 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)**.

eSens Listeria monocytogenes QT PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Sterile bilateral needle for vacuum tubes intended for venous blood collection for in vitro study.
- Vacuum blood collection system.
- Plastic container (50-60 ml) for storage and transportation of biological samples.
- Transport medium.
- 0.9 % of sodium chloride (sterile saline solution) or phosphate buffered saline (PBS) (137 mM sodium chloride; 2,7 mM potassium chloride; 10 mM sodium monophosphate; 2 mM potassium diphosphate; pH=7,5±0,2).
- Gynaecological combined probe.
- Flocked swab for collection, transportation and storage of biological samples.
- Reagent for whole blood pretreatment (selective lysis of erythrocytes).
- Sterile tools (individual for each sample) for homogenization (porcelain mortar and pestle) or homogenizer for pretreatment of tissue material.
- Vacuum aspirator with flask for removing supernatant.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 100 µl, 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 Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene tubes:

1. tightly closed 1.5-ml tubes for sampling.
2. screwed 50-ml tubes for sampling
3. screwed or tightly closed 1.5-ml or 2.0-ml tubes for pretreatment.
4. screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
5. thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
6. thin-walled 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 2-8 °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

NOTE: 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.

eSens Listeria monocytogenes QT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (whole blood, umbilical cord blood, cerebro-spinal fluid (CSF), node aspirates, swabs from the respiratory tract (oropharyngeal and nasopharyngeal swabs), swabs from the eye conjunctiva, amniotic fluid, placenta, swabs from vagina mucous membrane, urine, breast milk, meconium, faeces, autopsy material, environmental samples (concentrated water samples (wastewater, potable water, surface water)), liquid medium for primary enrichment of food product).

Sampling

6.1 Whole blood, umbilical cord blood

Blood should be taken after overnight fasting or in 3 hours after eating by a disposable 0.8-1.1 mm diameter needle into the tube (special vacuum system) with EDTA or sodium citrate solution as anticoagulant. After blood sampling the tube should be smoothly rotated several times for the thoroughly mixing with the anticoagulant. (Otherwise, blood will coagulate and DNA extraction will be impossible!). Place the tube in the rack after rotating.

Blood samples can be stored:

- at the temperature from 20 to 25 °C – for 6 hours after material sampling;
- at the temperature from 2 to 8°C – not to exceed one day.

Freezing of whole blood samples is unallowable!

6.2 Cerebro-spinal fluid (CSF)

Cerebrospinal fluid is collected in an amount no less than 1 ml using sterile needle into dry disposable 1.5-ml tubes.

The cerebrospinal fluid samples can be stored:

- at room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

6.3 Node aspirate

Node aspirate is obtained using sterile syringe and then poured into dry disposable 1.5-ml tubes.

The node aspirate samples can be stored:

- at the temperature from 18 to 25 °C – for 1 day;
- at the temperature from 2 to 8 °C – for 2 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

6.4 Oropharyngeal swabs

The material is taken with a sterile dry probe with a viscose tip. Rotate the probe over the tonsillar area, palatine arches, and posterior area of the oropharynx. When the material is obtained, place the probe (the working part with the cotton swab) into the sterile disposable tube containing transport medium and break off the plastic stick at the distance no more than 0.5 cm from the working part. If it is impossible to break off the working part of the probe, the biomaterial should be washed out of it to the tube with transport medium. To do this pin the working part inside the tube and rotate it clockwise and counterclockwise for 5-10 times. It is unacceptable to use scissors to cut the working part of the probe!

Tightly close the tube without gap between the cap and the tube and avoiding deformation of the interior part of the cap. Mark the tube.

The oropharyngeal swabs can be stored:

- at the temperature from 18 to 25 °C – for 1 day;
- at the temperature from 2 to 8 °C – for 2 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

6.5 Nasopharyngeal swabs

The material is taken with a sterile dry probe with a viscose tip. Insert the probe with a gentle motion over the lateral dorsum of the nose at the depth of 2-3 cm to the inferior nasal concha. Then slightly turn down the probe, insert into the inferior nasal meatus underneath the inferior nasal concha, rotate it and remove lateral dorsum of the nose. When the material is obtained, place the probe (the working part with the cotton swab) into the sterile disposable tube containing transport medium and break off the plastic stick at the distance no more than 0.5 cm from the working part. If it is impossible to break

off the working part of the probe, the biomaterial should be washed out of it to the tube with transport medium. To do this pin the working part inside the tube and rotate it clockwise and counterclockwise for 5-10 times. It is unacceptable to use scissors to cut the working part of the probe!

Tightly close the tube without gap between the cap and the tube and avoiding deformation of the interior part of the cap. Mark the tube.

The nasopharyngeal swabs can be stored:

- at the temperature from 18 to 25 °C – for 1 day;
- at the temperature from 2 to 8 °C – for 2 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

6.6 Swabs from the eye conjunctiva

The material is taken with a sterile dry probe under regional anesthesia (2 drops of dicain solution). Abduce the lower eyelid, rotate the probe 4-5 times over the surface of the eye conjunctiva, capturing the inner and outer corners of the eye. When the material is obtained, place the probe (the working part with the cotton swab) into the sterile disposable tube containing transport medium and break off the plastic stick. Leave the working part of the probe in the transport medium. If it is impossible to break off the working part of the probe, the biomaterial should be washed out of it to the tube with transport medium. To do this pin the working part inside the tube and rotate it clockwise and counterclockwise for 5-10 times. Tightly close the tube without gap between the cap and the tube and avoiding deformation of the interior part of the cap. Mark the tube.

The swabs from the eye conjunctiva can be stored:

- at the temperature from 18 to 25 °C – for 1 day;
- at the temperature from 2 to 8 °C – for 2 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

6.7 Amniotic fluid

Amniotic fluid is taken during amniocentesis test using disposable needles into the sterile disposable 1.5-ml tubes in an amount not less than 1.0 ml.

The samples of amniotic fluid can be stored:

- at room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

6.8 Autopsy material and placenta

The material should be taken from the proposed pathogen location, from the lesional tissue or the area surrounding the lesional tissue.

The tissue pieces (no more than 5 mm in a diameter) should be placed into the sterile disposable 1.5-ml tubes containing 0.1 ml of transport medium. Tightly close the tube without gap between the cap and the tube and avoiding deformation of the interior part of the cap.

The tissue pieces (more than 5 mm in a diameter) should be placed into the sterile disposable 50-ml vials with wide mouth and screw cap.

The samples of autopsy material and placenta can be stored:

- at room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

6.9 Swabs from vagina mucous membrane

The material should be obtained from the posteroinferior vaginal vault by the universal or cotton swab into a tube with transport medium. Rotate the working part of the swab over the surface of the lateral vaginal wall. Take the material from the vagina in sufficient quantity. The minimal presence of impurities such as mucus and blood is allowed. Transfer the swab into a tube with the transport medium. Break off the working part of the swab with the material and leave it in the tube with transport medium. If it is impossible to break off the working part of the probe, the biomaterial should be washed out of it to the tube with transport medium. To do this pin the working part inside the tube and rotate it clockwise and counterclockwise for 5-10 times. Tightly close the tube without gap between the cap and the tube and avoiding deformation of the interior part of the cap. Mark the tube. If the **Transport Medium with Mucolytic Agent** **REF** 952-CE; **REF** 953-CE is used its color can be changed due to the change of pH (then the discharge is acidic).

The swabs from vagina mucous membrane can be stored:

- at the temperature from 18 to 25 °C – for no more than 1 day;
- at the temperature from 2 to 8 °C – for no more than 2 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

6.10 Urine

The first portion of first void urine is taken for PCR-analysis in an amount of 20-30 ml into the dry sterile container (50-60 ml) or dry clean vessel

The urine samples can be stored:

- at room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C – for a long time

Only one freeze-thawing cycle is acceptable.

6.11 Breast milk

Breast milk is taken in an amount no less than 1.0 ml into sterile disposable 50-ml vials with wide mouth and screw caps.

The samples of breast milk can be stored:

- at room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;

- at the temperature not more than minus 68 °C – for a long time

Only one freeze-thawing cycle is acceptable.

6.12 Meconium and faeces

Meconium and faeces are taken in a small amount (4-6 times) from several locations using disposable spatula attached to the lid of the vial in which the material is taken.

The samples can be stored*:*

- at room temperature – for 6 hours;
- at the temperature from 2 to 8 °C - for 3 days;
- at the temperature from minus 24 to minus 16 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

6.13 Concentrated water samples

The water samples are obtained into the sterile disposable 50-ml vials with screw caps.

The samples can be stored*:*

- at room temperature – for 1 day;
- at the temperature from 2 to 8 °C – for 1 month;
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

6.14 *Liquid medium for primary enrichment of food product* according to state and local authorities' requirements regarding to the methods of detection and identification of pathogenic bacteria causing foodborne diseases, in food products by the polymerase chain reaction (PCR) with hybridization-fluorescence detection.

The samples of liquid medium for primary enrichment of food product can be stored:

- at the temperature from 18 to 25 °C – for no more than 1 day;
- at the temperature from 2 to 8 °C – for no more than 2 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

Pretreatment

6.15 Node aspirates, swabs from the respiratory tract, swabs from the eye conjunctiva, swabs from vagina mucous membrane, liquid medium for primary enrichment of food product. The pretreatment is not required.

6.16 The whole blood and umbilical cord blood samples

These samples need to to be pretreated. Add 1.0 ml of **Hemolytic** (REF 137-CE) and 0.25 ml of blood to the Eppendorf 1.5-ml tube. Gently vortex the tubes and leave them for 10 minutes, stirring occasionally. Centrifuge at 4,000 g (for example, 8,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 2 minutes. Remove the supernatant using vacuum aspirator without disturbing the pellet. After washing the cell pellet should be white, only a small pinkish bloom on the pellet is allowed (the remains of the destroyed erythrocytes). The washing using **Hemolytic** may be repeated if necessary. The obtained leucocytes pellet must be immediately lysed (in case of extraction using **RIBO-prep** add **300 µl of Solution for Lysis** and then extract DNA in accordance with the *Instruction Manual* enclosed to

the RIBO-prep reagent kit without adding **Solution for Lysis** once again) or frozen at the temperature not more than minus 68 °C for a long time.

6.17 Cerebro-spinal fluid samples are to be pretreated.

Transfer 1 ml of the material using the filter tip into the sterile disposable 1.5-ml tube. Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 min. After centrifugation carefully remove the supernatant leaving 100 µl of it. Resuspend the material on the vortex.

The cerebrospinal fluid samples can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – for 1 day,
- at the temperature from minus 24 to minus 16 °C – for 1 month,
- at the temperature not more than minus 68 °C – for a long time.

6.18 The samples of amniotic fluid are to be pretreated.

Thoroughly resuspend the sample of amniotic fluid on vortex. Transfer 1 ml of material into the 1.5-ml tube using filter tip. Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 min. After centrifugation carefully remove the supernatant leaving 100 µl of it. Resuspend the material on the vortex.

The pretreated material can be stored:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C – for a long time.

6.19 The samples of breast milk are to be pretreated.

Mix the breast milk by pipetting. Transfer 1 ml of material into the 1.5-ml tube using filter tip. Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 min. After centrifugation carefully remove the supernatant leaving 100 µl of it. Resuspend the material on the vortex.

The pellet of breast milk can be stored:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C – for a long time.

6.20 *The urine samples* are to be pretreated.

Shake the vial with urine. Transfer 1 ml of urine into the sterile disposable 1.5-ml tube using filter tip. Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 min. In case of large amount of salts resuspend only 1 ml of upper lower of salt pellet and then concentrate again. Completely remove the supernatant using vacuum aspirator with trap flask without disturbing the pellet. Add **Transport medium with mucolytic agent** (**REF**952-CE; **REF** 953-CE) to the finale volume 0.2 ml, thoroughly vortex.

The pretreated urine samples can be stored before the PCR-analysis:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C – for a long time.

6.21 The samples of meconium and faeces are to be pretreated.

Transfer 0.8 ml of phosphate buffer (sterile isotonic sodium chloride solution) into the sterile disposable 1.5-ml tube. Using a new one filter tip add 0.1 g (0.1 ml) of meconium/faeces and thoroughly vortex to prepare homogeneous suspension. Centrifuge at 7,000-12,000 g (for example, 10,000-13,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 minutes. Using a new one filter tip transfer 0.05 ml of bacterial particle (the upper white-yellow lower of the pellet with high concentration of bacterium) to the tube with 0.8 ml of phosphate buffer (sterile isotonic sodium chloride solution). If the pellet or the white-yellow boundary lower between the pellet and supernatant are absent, take 0.1 ml from the bottom of the tube or from the border between the pellet and supernatant respectively. Thoroughly vortex the pellet and centrifuge at 7,000-12,000 g (for example, 10,000-13,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 minutes. Remove the supernatant, add 0.3 ml of phosphate buffer (sterile isotonic sodium chloride solution) and resuspend on the vortex.

6.22 The samples of autopsy material and placenta are to be pretreated.

Place the autopsy material or placenta (tissue pieces of a diameter more than 5 mm) to the sterile mortar and homogenate using the pestle. Add sterile isotonic sodium chloride solution to the prepared homogenate and mix thoroughly. Use the obtained suspension for DNA extraction.

Pretreatment is not required for autopsy material and placenta (tissue pieces of a diameter no more than 5 mm) placed in the tubes with transport medium.

The samples can be stored:

- at room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 week;
- at the temperature not more than minus 68 °C – for a long time.

6.23 The concentrated water samples.

The pretreatment is not required. In case of visible impurities or color in the sample thoroughly mix it before the analysis and then transfer into the 1.5- or 2.0-ml tube. Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 1 min. Use supernatant for DNA extraction.

The material can be stored:

- at the temperature from 2 to 8 °C – for no more than 1 day;
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

Interfering substances and limitations of using test material samples

The excessive amount of impurities in biological material such as mucus, blood, pus, and others can lead to the amplification reaction inhibition. In order to control the RNA/DNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control STI-87 (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

The next samples are inapplicable for analysis:

- the urine samples collected more than 24 hours before the delivery to the laboratory,
- the whole blood samples, collected in the tubes with heparin as anticoagulant,
- the whole blood samples, containing blood clot or which has been exposed to freezing.

7 WORKING CONDITIONS

eSens Listeria monocytogenes QT PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction:

- **RIBO-prep**, (K2-9-Et-50-CE)

- For the automatic extraction:

- **ePure Bacterial DNA Extraction Kit** (E2006)

The DNA extraction of each test sample is carried out in the presence of **Internal Control STI-87 (IC).**

Add **10 µl** of **Internal Control STI-87 (IC)** to each tube with samples.

The volume of the test sample is **100 µl.**

In the extraction procedure it is necessary to carry out the control reactions as follows:

C–	Add **100 µl of Negative Control (C–)** to the tube labelled **C–** (Negative Control of Extraction).
PCE	Add **90 µl of Negative Control (C–)** and **10 µl of Positive Control *Listeria monocytogenes*** to the tube labeled **PCE** (Positive Control of Extraction).

The volume of elution is **50 µl.**

NOTE: Extract DNA according to the manufacturer's protocol.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

1. Calculate the required quantity of each reagent for one reaction:

- **10 µl of PCR-mix-FL Listeria monocytogenes**,
- **5 µl of PCR-buffer-H**.

Prepare the reaction mixture for the total number of test and control samples according to the Table 2.

NOTE: Prepare the reaction mixture just before the use.

Table 2

Scheme of reaction mixture preparation

Reagent volume per 1 reaction (µl)			**10,0**	**5,0**
Number of samples			**Reagent volume per specified number of reactions (µl)**	
for quantitative detection	**for qualitative detection**	**Number of reactions***	**PCR-mix-FL *Listeria monocytogenes***	**PCR-buffer-H**
1	**4**	**9**	**90**	**45**
2	**5**	**10**	**100**	**50**
3	**6**	**11**	**110**	**55**
4	**7**	**12**	**120**	**60**
5	**8**	**13**	**130**	**65**
6	**9**	**14**	**140**	**70**
7	**10**	**15**	**150**	**75**
8	**11**	**16**	**160**	**80**
9	**12**	**17**	**170**	**85**
10	**13**	**18**	**180**	**90**
11	**14**	**19**	**190**	**95**
12	**15**	**20**	**200**	**100**
13	**16**	**21**	**210**	**105**
14	**17**	**22**	**220**	**110**
15	**18**	**23**	**230**	**115**
16	**19**	**24**	**240**	**120**
17	**20**	**25**	**250**	**125**
18	**21**	**26**	**260**	**130**
19	**22**	**27**	**270**	**135**
20	**23**	**28**	**280**	**140**
21	**24**	**29**	**290**	**145**
22	**25**	**30**	**300**	**150**

Reagent volume per 1 reaction (µl)			**10,0**	**5,0**
Number of samples			**Reagent volume per specified number of reactions (µl)**	
for quantitative detection	**for qualitative detection**	**Number of reactions***	**PCR-mix-FL *Listeria monocytogenes***	**PCR-buffer-H**
23	**26**	**31**	**310**	**155**
24	**27**	**32**	**320**	**160**
25	**28**	**33**	**330**	**165**
30	**33**	**34**	**340**	**170**

2. Thaw the tube with **PCR-mix-FL *Listeria monocytogenes*.** Thoroughly vortex the tubes with **PCR-mix-FL *Listeria monocytogenes*, PCR-buffer-H** and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL *Listeria monocytogenes* and PCR-buffer-H**. Sediment the drops by vortex.
4. Take the required number of tubes or strips for amplification for the clinical and control samples. The type of tubes depends on the PCR instrument used for analysis.
5. Transfer **15 µl** of the prepared mixture to each tube. Discard the unused reaction mixture.
6. Add **10 µl** of **DNA samples** extracted from test samples of DNA extraction stage.
7. Carry out the 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 **C+** (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 Listeria monocytogenes reagent** to the tube labeled **PCE** (Positive control of Extraction).

for quantitative analysis:

NCA	Add **10 µl of TE-buffer** to the tube labeled **NCA** (Negative Control of Amplification)
C1 LIM	Add **10 µl of DNA calibrator C1 LIM** into two tubes labeled **C1**
C2 LIM	Add **10 µl of DNA calibrator C2 LIM** into two tubes labeled **C2**
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 Listeria monocytogenes reagent** to the tube labeled **PCE** (Positive control of Extraction).

* For quantitative analysis: number of reactions = number of test samples (N) + controls of Extraction (C-, PCE) and Amplification (DNA-calibrators C1 LIM, C2 LIM in two repeats, NCA) + one extra sample

(N+7+1).
For qualitative analysis: number of reactions number of test samples (N) + controls of Extraction (C-, PCE) and Amplification (DNA-calibrator C2 LIM, NCA) + one extra sample (N+4+1).

NOTE: Carry out the PCR just after the mix of reaction mixture and DNA-samples and controls.

8.2.2 Amplification

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

Table 3

Unified amplification and detection program for rotor-type (e.g Rotor-Gene Q or equivalent) and plate-type (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.) instruments

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
3	95	10 s	–	45
	60	20 s	**FAM, JOE, ROX**	

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

NOTE: Any combination of the tests (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in "multiprime" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If in one instrument only the tests for the pathogen DNA detection are carried out simultaneously, the first step of reverse transcription (50 °C – 15 min) can be omitted for time saving.

2. Adjust the fluorescence channel sensitivity according to *Technical sheet*.
3. Insert tubes into the reaction module of the device.

NOTE: Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

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:

Table 4

Channel for the fluorophore	FAM	JOE	ROX
For whole blood, umbilical cord blood, placenta, swabs from vagina mucous membrane, node aspirates and autopsy material			
Signal registration, indicating the amplification product accumulation	Internal Control STI-87 DNA	*Listeria monocytogenes* DNA	Internal Control Glob DNA
For amniotic fluid, breast milk, cerebro-spinal fluid (CSF), swabs from the respiratory tract, swabs from the eye conjunctiva, urine, meconium, faeces, liquid medium for primary enrichment of food product and concentrated water samples			
Signal registration, indicating the amplification product accumulation	Internal Control STI-87 DNA	*Listeria monocytogenes* DNA	not taking into account

Results are interpreted by the crossing (or not-crossing) the S-shaped (sigmoid) 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.

Qualitative analysis

The principle of interpretation is the following:

Table 5

Results interpretation for the test samples (qualitative analysis)

***Ct* value in the channel for the fluorophore**			**Result**
FAM	**JOE**	**ROX**	
For whole blood, umbilical cord blood, placenta, swabs from vagina mucous membrane, node aspirates and autopsy material			
< boundary value	absent	< boundary value	*L. monocytogenes* DNA **is not detected**
< boundary value	< boundary value	< boundary value	*L. monocytogenes* DNA **is detected**
absent or > boundary value	absent or > boundary value	absent or > boundary value	**Invalid result***
< boundary value	> boundary value	< boundary value	**Equivocal****
For amniotic fluid, breast milk, cerebro-spinal fluid (CSF), swabs from the respiratory tract, swabs from the eye conjunctiva, urine, meconium, faeces, liquid medium for primary enrichment of food product and concentrated water samples			
< boundary value	absent	—	*L. monocytogenes* DNA **is not detected**
< boundary value	< boundary value	—	*L. monocytogenes* DNA **is detected**
absent or > boundary value	absent or > boundary value	—	**Invalid result***
< boundary value	< boundary value	—	**Equivocal****

* In case of **invalid result**, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage.

** In case of **equivocal result**, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage. If the same result is obtained, the sample is considered positive. If the negative result is obtained in the second run, the sample is considered equivocal and re-sampling of the material for analysis is recommended

NOTE: Boundary *Ct* values are specified in the *Technical sheet* enclosed to the PCR kit.

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 Negative Control of extraction are correct (see Table 6 and the *Technical sheet* enclosed to the PCR kit).

Table 6

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore		
		FAM	JOE	ROX
For whole blood, umbilical cord blood, placenta, swabs from vagina mucous membrane, node aspirates and autopsy material				
PCE	DNA extraction	<boundary value	<boundary value	<boundary value
C–	DNA extraction	<boundary value	Absent	absent or > boundary value
NCA	PCR	Absent	Absent	Absent
C+	PCR	<boundary value	<boundary value	<boundary value
For amniotic fluid, breast milk, cerebro-spinal fluid (CSF), swabs from the respiratory tract, swabs from the eye conjunctiva, urine, meconium, faeces, liquid medium for primary enrichment of food product and concentrated water samples				
PCE	DNA extraction	<boundary value	<boundary value	–
C–	DNA extraction	<boundary value	Absent	–
NCA	PCR	Absent	Absent	–
C+	PCR	<boundary value	<boundary value	–

Quantitative analysis

Based on the obtained *Ct* values and specified concentration values of DNA calibrators (C1 and C2) a calibration line is plotted and the number of copies of *L. monocytogenes* DNA, human DNA and Internal Control STI-87 per 1ml of test and control samples is calculated. Obtained values are used for calculation of *L. monocytogenes* DNA quantity per 1 ml of the sample according to the formula:

number of *L. monocytogenes* DNA in 1 ml x A = copies/ml

where:

A – the coefficient taking into account the volume of extraction is calculated according to the formula:

$$\textbf{Coefficient A} = \frac{\textbf{100}}{\textbf{extraction volume, µl}}$$

NOTE: Concentration values of DNA-calibrators are specified in the *Technical sheet* enclosed to the PCR kit.

Table 7

Results interpretation for the test samples (quantitative analysis)

Result	**Interpretation**
For whole blood, umbilical cord blood, placenta, swabs from vagina mucous membrane, node aspirates and autopsy material	
Invalid	The *Ct* value in the channel for the FAM channel is absent or greater than the boundary *Ct* value, whereas the *Ct* value determined in the channel for the ROX fluorophore is greater than the boundary *Ct* value. Moreover, the calculated concentrations of *L. monocytogenes* DNA fall in the linear measurement range of the PCR kit. The PCR analysis should be repeated starting from the DNA extraction stage. If the *Ct* value in the channel for ROX fluorophore is absent, the sampling of biological material and PCR-analysis should be repeated.
L. monocytogenes DNA is not detected	The *Ct* value for *L.monocytogenes* DNA is absent and the *Ct* value determined in the channels for FAM and ROX fluorophores is less than the boundary *Ct* value. The result is *Listeria monocytogenes* DNA is not detected.
For amniotic fluid, breast milk, cerebro-spinal fluid (CSF), swabs from the respiratory tract, swabs from the eye conjunctiva, urine, meconium, faeces, liquid medium for primary enrichment of food product and concentrated water samples	
Invalid	The *Ct* value in the channel for FAM fluorophore is absent or greater than the boundary *Ct* value, whereas the calculated concentrations of *L. monocytogenes* DNA fall in the linear measurement range of the PCR kit. The PCR analysis should be repeated starting from the DNA extraction stage.
L. monocytogenes DNA is not detected	The *Ct* value for *L.monocytogenes* DNA is absent and the *Ct* value determined in the channel for FAM fluorophore is less than the boundary *Ct* value. The result is *Listeria monocytogenes* DNA is not detected.
< 1x10^{3} copies/ml	*L. monocytogenes* DNA is detected in the concentration less than the lower limit of the linear measurement range of the PCR kit. The result is less than 1x10^{3} *Listeria monocytogenes* DNA copies/ml.
Xx10^{y} copies/ml	The calculated concentration value (copies/ml) falls in the linear measurement range of the PCR kit. The result is *Listeria monocytogenes* DNA is detected in the concentration Xx10^{y} copies/ml.
> 1x10^{8} copies/ml	*L. monocytogenes* DNA is detected in the concentration more than the upper limit of the linear measurement range of the PCR kit. The result is more than 1x10^{8} *Listeria monocytogenes* DNA copies/ml.

NOTE: Boundary *Ct* values are specified in the *Technical sheet* enclosed to the PCR kit.

The result of the quantitative 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 8 and the *Technical sheet* enclosed to the PCR kit).

Table 8

Results for controls

<table>
<tr><th rowspan="2">Control</th><th rowspan="2">Stage for control</th><th colspan="3">Result of amplification in the channel for fluorophore</th></tr>
<tr><th>FAM</th><th>JOE</th><th>ROX</th></tr>
<tr><td colspan="5">For whole blood, umbilical cord blood, placenta, swabs from vagina mucous membrane, node aspirates and autopsy material</td></tr>
<tr><td>PCE</td><td>DNA extraction</td><td>< boundary Ct value</td><td>< boundary Ct value; concentration value falls in the range</td><td>< boundary Ct value</td></tr>
<tr><td>C–</td><td>DNA extraction</td><td>< boundary Ct value</td><td>Absent</td><td>absent or > boundary Ct value</td></tr>
<tr><td>NCA</td><td>PCR</td><td>Absent</td><td>Absent</td><td>Absent</td></tr>
<tr><td>C1</td><td>PCR</td><td>Defined</td><td>Defined</td><td>Defined</td></tr>
<tr><td>C2</td><td>PCR</td><td>< boundary Ct value</td><td>< boundary Ct value</td><td>< boundary Ct value</td></tr>
<tr><td colspan="5">For amniotic fluid, breast milk, cerebro-spinal fluid (CSF), swabs from the respiratory tract, swabs from the eye conjunctiva, urine, meconium, faeces, liquid medium for primary enrichment of food product and concentrated water samples</td></tr>
<tr><td>PCE</td><td>DNA extraction</td><td>< boundary Ct value</td><td>< boundary Ct value; concentration value falls in the range</td><td>-</td></tr>
<tr><td>C–</td><td>DNA extraction</td><td>< boundary Ct value</td><td>Absent</td><td>-</td></tr>
<tr><td>NCA</td><td>PCR</td><td>Absent</td><td>Absent</td><td>-</td></tr>
<tr><td>C1</td><td>PCR</td><td>Defined</td><td>Defined</td><td>-</td></tr>
<tr><td>C2</td><td>PCR</td><td>< boundary Ct value</td><td>< boundary Ct value</td><td>-</td></tr>
</table>

10 TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of amplification (C+) in the channels for the FAM and/or JOE and/or ROX fluorophores is greater than the boundary *Ct* value or absent. The amplification and detection should be repeated for all the samples.
2. The *Ct* value determined for the Positive Control of Extraction (PCE) in the channels for the FAM and/or JOE and/or ROX fluorophores is greater than the boundary *Ct* value specified in the *Technical sheet* or absent. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.

3. For quantitative analysis, the calculated concentration of the Positive control *Listeria monocytogenes* does not fit in the range specified in the *Technical sheet*. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.
4. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the channel for the JOE fluorophore and the *Ct* value determined in the channel for the ROX fluorophore is less than the boundary value. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
5. If the *Ct* value is determined for the Negative Control of amplification (NCA) in any of the channels for the FAM and/or JOE and/or ROX fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific DNA was detected.
6. For quantitative analysis, the *Ct* value is absent for the DNA-calibrator C1 in the specified detection channels (see Table 7). The amplification and detection should be repeated for all the samples.
7. For quantitative analysis, the *Ct* value is absent or is greater than the boundary value for the DNA-calibrator C2 in the specified detection channels. The amplification and detection should be repeated for all the samples.
8. The correlation coefficient R^2 is less than 0.98 when plotting the calibration curve. Check the correctness of set concentrations of calibrators in accordance with the *Technical sheet*. If the improper result has been obtained again the amplification and detection for all the samples should be repeated.
9. If the *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

11 TRANSPORTATION

eSens Listeria monocytogenes QT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens Listeria monocytogenes QT PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *Listeria monocytogenes* and PCR-buffer-H). All components of the **eSens Listeria monocytogenes QT 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.

NOTE: PCR-mix-FL *Listeria monocytogenes* and PCR-buffer-H are to be stored at the temperature from minus 24 °C to minus 16 °C.

NOTE: PCR-mix-FL *Listeria monocytogenes* is to be kept away from light.

13 SPECIFICATIONS

13.1 Analytical sensitivity

The analytical sensitivity of **eSens Listeria monocytogenes QT PCR kit** is the following:

Biological material	Sample volume for extraction, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity, copies/ml	Linear measurement range, copies/ml
Whole blood, umbilical cord blood, cerebro-spinal fluid (CSF), node aspirates, swabs from the respiratory tract, swabs from the eye conjunctiva, amniotic fluid, swabs from vagina mucous membrane, urine, breast milk, meconium, faeces, environmental samples (concentrated water samples), liquid medium for primary enrichment of food product	100	RIBO-prep	eSens Listeria monocytogenes QT PCR kit	$5x10^2$	$1x10^3 – 1x10^8$

The claimed features are achieved while respecting the rules specified in the section "*Sampling and Handling*".

13.2 Analytical specificity

The analytical specificity of **eSens Listeria monocytogenes QT 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.

The PCR kit detects the DNA fragments of *L. monocytogenes*. Specific activity of the kit was defined by investigation of the following strains of *L.monocytogenes*. Analytical specificity was proved in studies of DNA/RNA of the following microorganisms: *Escherichia coli*, *Staphylococcus saprophyticus*, methicillin-resistant *Staphylococcus aureus* (*MRSA*), *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *Streptococcus milleri*, *Streptococcus sanguinis*, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Acinetobacter baumanii*, *Proteus mirabilis*, *Haemophilus influenzae*, *Haemophilus haemolyticus*, *Haemophilus parasuis*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Lactobacillus* spp., *Neisseria subflava*, *Neisseria cinerea*, *Neisseria mucosa*, *Neisseria gonorrhoeae*, *Neisseria sicca*, *Neisseria flavescens*, *Neisseria meningitidis*, *Neisseria elongata*, *Candida albicans*, *Candida glabrata*, *Cryptococcus neoformans*, *Toxoplasma gondii*, *Herpes simplex virus* I (*HSV* I), *Herpes simplex virus* II (*HSV* II), *Cytomegalovirus hominis (CMV)*, *Epstein-Barr virus (EBV)*, *Human herpesvirus 6 (HHV-6)*, *Human herpesvirus 7 (HHV-7)*, *Varicella-zoster virus (VZV)*, *JC virus (JCV)*, *BK virus (BKV)*, *Parvovirus* B19, *Enterovirus* and human DNA.

While testing the DNA/RNA of above-mentioned microorganisms and human DNA nonspecific reactions were absent.

13.3 Reproducibility, repeatability and trueness

The reproducibility and repeatability were determined by testing the dilutions of quality control sample (QCS) in biological material samples.

Table 9

Reproducibility

Microorganism	Initial concentration value, copies/ml	Number of repeats	Average concentration value, lg	Standard deviation (SD)	The coefficient of variation (CV), %
L. monocytogenes	$1x10^6$	48	5.97	0.06	1.07
L. monocytogenes	$5x10^4$	48	4.66	0.13	2.76
L. monocytogenes	$1x10^3$	48	3.04	0.17	5.49

Table 10

Repeatability

Microorganism	Initial concentration value, copies/ml	Number of repeats	Average concentration value, lg	Standard deviation (SD)	The coefficient of variation (CV), %
L. monocytogenes	$1x10^6$	16	5.85	0.07	1.26
L. monocytogenes	$5x10^4$	16	4.66	0.06	1.23
L. monocytogenes	$1x10^3$	16	3.05	0.21	7.00

Table 11

Trueness

Microorganism	Number of repeats	Average value of measurement, lg	Specified value, lg	Bias (B), %
L. monocytogenes	100	8.02	8.00	0.25

The clinical specificity of **eSens Listeria monocytogenes QT PCR kit** was confirmed in laboratory clinical trials.

13.4 Diagnostic characteristics

Table 12

The results of testing eSens Listeria monocytogenes QT PCR kit in comparison with the reference assay

Samples type	The results of application of eSens Listeria monocytogenes QT PCR kit		Results of using the reference assay	
			Positive	Negative
Listeria monocytogenes strain (ATCC® 7644™)	60 samples were tested	Positive	60	0
		Negative	0	0

Table 13

Effectiveness of using eSens Listeria monocytogenes QT PCR kit

Samples type	Biological material containing *L. monocytogenes* DNA fragment			Biological material not containing *L. monocytogenes* DNA fragment		
	Number of samples	Result		Number of samples	Result	
		Positive	Negative		Positive	Negative
whole blood	100	100	0	100	0	100
umbilical cord blood	100	100	0	100	0	100
cerebro-spinal fluid (CSF)	100	100	0	100	0	100
node aspirates	100	100	0	100	0	100
swabs from the respiratory tract	100	100	0	100	0	100
swabs from the eye conjunctiva	100	100	0	100	0	100
amniotic fluid	100	100	0	100	0	100
swabs from vagina mucous membrane	100	100	0	100	0	100
urine	100	100	0	100	0	100
breast milk	100	100	0	100	0	100
meconium	100	100	0	100	0	100
faeces	100	100	0	100	0	100

Samples type	Biological material containing *L. monocytogenes* DNA fragment			Biological material not containing *L. monocytogenes* DNA fragment		
	Number of samples	Result		Number of samples	Result	
		Positive	Negative		Positive	Negative
liquid medium for primary enrichment of food product	100	100	0	100	0	100
environmental samples (concentrated water samples)	100	100	0	100	0	100
placenta	100	100	0	100	0	100
autopsy material	100	100	0	100	0	100

The samples of different biological material type containing dilutions of quality control samples at the concentration no more than $1x10^4$ were used as biological material containing *L. monocytogenes* DNA fragment.

Table 14

Diagnostic characteristics of eSens Listeria monocytogenes QT PCR kit

Sample type	**Nucleic acid extraction kit**	**Diagnostic sensitivity (Relative sensitivity in comparison with the used reference assay),** **no less than %**	**Diagnostic specificity (Relative specificity in comparison with the used reference assay),** **no less than %**
Whole blood, umbilical cord blood, cerebro-spinal fluid (CSF), node aspirates, swabs from the respiratory tract, swabs from the eye conjunctiva, amniotic fluid, swabs from vagina mucous membrane, urine, breast milk, meconium, faeces, environmental samples (concentrated water samples), liquid medium for primary enrichment of food product	RIBO-prep	100	100

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

KEY TO SYMBOLS USED

Symbol	Meaning	Symbol	Meaning
REF	Catalogue number		Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
IVD	In vitro diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	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
01_04/2022		

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