DNA-TECHNOLOGY

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RUO

For research use only

Listeria monocytogenes

REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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R1-P003-23/4EU
R1-P003-UA/9EU

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1. INTENDED USE

The **Listeria monocytogenes REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Listeria monocytogenes REAL-TIME PCR Detection Kit** is designed for detection of *Listeria monocytogenes* DNA in human biological material (blood, scrapes from mucous membranes, cerebrospinal fluid, amniotic fluid, biopsy specimens or punctate from lesions of organs and tissues, feces or meconium) by real-time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Listeria monocytogenes REAL-TIME PCR Detection Kit.**

The **Listeria monocytogenes REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods and rules of work in the laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

Method: real-time polymerase chain reaction; qualitative analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent completion of the polynucleotide chains from these primers with Taq-polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a “hot” start is provided. For package S, “hot” start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. “Hot” start for package U is provided by using polymerase which activity is blocked by antibodies. The activation of the enzyme occurs only after preheating the reaction mixture at 94 °C. This eliminates the nonspecific annealing of primers on the DNA target during the initial heating of the test tube.

DNA probes containing a fluorescent label and a fluorescence quencher are introduced into the PCR-mix. When a specific product is formed, the DNA probe is destroyed and the quencher stops affecting the fluorescent label, which leads to an increase in the fluorescence level. The number of destroyed probes (and hence the fluorescence level) increases in proportion to the number of specific amplicons formed. The fluorescence level is measured at each amplification cycle in real time.

PCR-mix includes internal control (IC) designed to control the polymerase chain reaction.

The DNA probes for the detection of the DNA amplification product contain fluorescent dye Fam. The DNA for the detection of internal control amplification product contain fluorescent dye Hex.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam/Green	Hex/Yellow/Vic	Rox/Orange	Cy5/Red	Cy5.5/Crimson
Listeria monocytogenes	IC*	-	-	-

* — internal control

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime real-time thermal cyclers for **Listeria monocytogenes REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **Listeria monocytogenes REAL-TIME PCR Detection Kit** is also approved for use with Rotor-Gene (Qiagen), CFX96 (Bio-Rad) and Applied Biosystems Quant Studio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers.

3. CONTENT

The detailed description of content is represented in Tables 2-4.

Table 2. The **Listeria monocytogenes REAL-TIME PCR Detection Kit** content, package S, strips, for R1-P003-S3/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	960 µL (20 µL in each)	tubes, 6 strips of 8
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control[1]	Colorless transparent liquid	75 µL	1 tube
Strip caps	6 strips of 8		

Table 3. The **Listeria monocytogenes REAL-TIME PCR Detection Kit** content, package S, tubes, for R1-P003-23/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	960 µL (20 µL in each)	48 individual tubes
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control[1]	Colorless transparent liquid	75 µL	1 tube

Table 4. The **Listeria monocytogenes REAL-TIME PCR Detection Kit** content, package U, for R1-P003-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or pink transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
PCR-buffer	Colorless transparent liquid	600 µL	1 tube
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

All components are ready to use and do not require additional preparation for operation.

The **Listeria monocytogenes REAL-TIME PCR Detection Kit** (package S) is designed for 48 tests (no more than 12 runs), including analysis of test samples, negative controls and positive controls.

The **Listeria monocytogenes REAL-TIME PCR Detection Kit** (package U) is designed for 96 tests with at least 5 samples per run (3 test samples, negative control and positive control).

[1] - can be marked as C+

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

– Sterile containers to collect clinical material;

- For blood collection: 4.5 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL.

Please use only salt of EDTA as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 12,000);
- Solid-state thermostat (temperature range 50-65 °C);
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Electric laboratory aspirator with trap flask to remove supernatant;
- RNase and DNase free pipette tips for electric laboratory aspirator;
- Single channel pipettes (dispensers covering 20-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL; 1,000 µL);
- Pipette stand;
- Nucleic acid extraction kit ("DNA-Technology" made, **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU, **PREP-MB RAPID** REF P-116-A/8EU, **PREP-RAPID** REF P-001/1EU, **PREP-MB MAX** REF P-103-N/4EU, P-103-A/8EU, **PREP-OPTIMA** REF P-016-1/2EU, REF P-016-N/2EU are recommended);
- Physiological saline solution 0.9% NaCl (sterile);
- Transport medium (STOR-F manufactured by DNA-Technology is recommended);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator with freezer;
- Vortex mixer;
- Vortex rotor for 0.2 ml strips (in case of using package S, strips);
- Tube rack for 0.2 mL tubes;
- PCR tube rack for 0.2 mL strips;
- Tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1,000 µL volume range);

- RNase and DNase free filtered pipette tips (volume 20 µL)
- RNase and DNase free tips for semi-automatic pipettes (volume 200 µL; 1,000 µL);
- Pipette stand;
- RNase and DNase free 1.5 mL microfuge tubes with caps;
- RNase and DNase free 0.2 mL PCR tubes or 96-well microplate;
- Magnetic homogenizer (for **PREP-MB RAPID** and **PREP-MB MAX** extraction kits);
- DTstream *M1 dosing device ("DNA-Technology", LLC) (only for automated dosing in case of using package U);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream *M1 dosing device (only for automated dosing in case of using package U);
- Device for plate sealing DTpack ("DNA-Technology", LLC) (only for automated dosing in case of using package U);
- Centrifuge for microplates (RCF(g) at least 100) (only for automated dosing in case of using package U);
- Polymer thermal film for microplate sealing (in case of using package U);
- 384-well microplate (only for automated dosing in case of using package U);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-amplification – amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

5.1. Transport and storage conditions. Package S

- All components of the reagent kit must be stored in a refrigerator or a freezer at the temperature from 2 °C to 8 °C throughout the shelf life of the kit.
- Paraffin sealed PCR-mix must be kept away from light.
- Transport of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the components included in the kit.
- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.

5.2. Transport and storage conditions. Package U

- All components of the reagent kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a freezer at the temperature from 2 °C to 8 °C throughout the shelf life of the kit.
- PCR-mix must be kept away from light.
- TechnoTaq MAX polymerase must be stored in a freezer at the temperature from minus 18 °C to minus 22 °C throughout the shelf life of the kit.
- Transport of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the components included in the kit.
- It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container up to 25 °C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

5.3.1. Package S

- All components of the kit must be stored in a refrigerator or a freezer at temperatures from 2 °C to 8 °C during the storage period
- Paraffin sealed PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period

5.3.2. Package U

- All components of the kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a freezer at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 18 °C to minus 22 °C during the storage period.

WARNING! The kits stored under undue regime must not be used.

An expired **Listeria monocytogenes REAL-TIME PCR Detection Kit** must not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **Listeria monocytogenes REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular research are allowed to work with the kit.

Handle and dispose of all biological samples, reagents and materials used to carry out the test as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA**.** Avoid direct contact with the biological samples reagents and materials used to carry out the test. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Work surfaces, as well as rooms where PCR is performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work. Waste materials are

disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.
Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **Listeria monocytogenes REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from blood, scrapes of mucous membranes, cerebrospinal fluid, amniotic fluid, biopsy specimens or punctate from lesions of organs and tissues, feces or meconium.

7.1. Interfering substances

The presence of PCR inhibitors in a sample may cause doubtful (uncertain/unreliable) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product amplification.

PCR inhibitors include the following substances: hemoglobin present in the DNA sample as a result of incomplete removal of blood admixture from the sample, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of wash solutions during sample preparation.
Maximum concentrations of interfering substances at which amplification of laboratory and internal controls was not influenced: hemoglobin — 0.35 mg/mL of DNA sample, isopropyl alcohol — 100 µL/mL of DNA sample, methyl acetate — 100 µL/mL of DNA sample.
Admixtures contained in the biomaterial samples are almost fully removed during DNA extraction.
To reduce the number of PCR inhibitors, follow the rules of biomaterial sampling. If a large number of PCR inhibitors are suspected in the sample, it is recommended to choose DNA extraction methods that maximize the removal of PCR inhibitors from the sample; express methods of DNA extraction are not recommended.

7.2. General requirements

PCR analysis is a direct method of laboratory analysis, thus human biomaterial sample collection must be performed from the lesion of infection. The decision on studying a lesion shall be taken by a healthcare professional based on the collected anamnesis and the clinical picture of the disease.

Quality of biomaterial sample collection, its transport, storage and pretreatment are crucial for obtaining correct results.

If biomaterial must be taken from several biotopes, repeat the procedure taking material into a new tube each time.

Incorrect biomaterial sampling may lead to unreliable results and, thus, to the necessity of repeated sampling.

At the biomaterial preparation stage use RNase and DNase free filter tips.

To prevent contamination, only open the cap of the tube where biomaterial is introduced, and close it before proceeding to the next tube.

7.3. Sample collection

WARNING! Before DNA extraction, pre-processing of biological material samples may be needed.

Peripheral blood

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

Method limitations: intravenous heparin injections, infusions of parenteral nutrition less than 6 hours before the test.

Mucous scrapes (scrapes of epithelial cells from respiratory and urogenital tract)

WARNING! Material collection into tubes with “PREP-RAPID” reagent must be performed with dry swab. Avoid contact of solution with skin, eyes and mucous membranes.

Method limitations: for urogenital scrapes — local application of medicines and lubricants, vaginal ultrasound less than 24 hours before the analysis;

for respiratory scrapes — local application of medicines (sprays, drops, creams, ointments) less than 24 hours before the analysis. If using aerosols and other medicines for bronchial asthma inhalations, please sample material for the analysis no earlier than three hours after inhalation.

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

Urogenital scrapes

Women must not perform vaginal douching the day before the analysis. To obtain a reliable result, biomaterial must contain the highest possible number of epithelial cells and a minimum amount of mucus and blood.

WARNING! Before obtaining a scrape of epithelial cells from the urethra, the free-flowing secretion should be removed with a sterile cotton swab.

If biomaterial must be taken from several biotopes, repeat the procedure taking material into a new tube each time.

Vaginal samples

The sample must be taken prior to manual inspection. Speculum can be treated with hot water before the procedure. Antiseptics must not be used for speculum treatment. The sample must be taken from posterolateral vaginal wall. In girls, the material is taken from the mucous membrane of the vaginal vestibule, and in some cases from the posterior vaginal vault through the hymenal rings.

Urethral samples

Urination 1.5-2 hours prior to sampling procedure is prohibited.

Immediately prior to the sampling procedure, the external urethral orifice must be treated with a swab moistened with sterile physiological saline solution.

In case of purulent discharge, the sample must be taken 15-20 minutes after urinating. In the absence of discharge, it is necessary to massage urethra with sampling swab or brush. Carefully insert the swab into the woman’s urethra to a depth of 1-1.5 cm. A child’s sample must be taken from the external urethral orifice.

Cervical canal samples

Before sampling remove mucus with a swab and treat the cervix with sterile physiological saline solution. Carefully insert sampling swab into the cervix to a depth of 0.5-1.5 cm. Avoid contact with vaginal wall when removing the swab.

Cerebrospinal fluid, amniotic fluid, feces, meconium

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

Biopsy specimens/punctate

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

If using PREP-NA and PREP-GS reagent kits for DNA extraction:

Collect samples into 1.5 mL tubes with transport medium designed for transport and storage of PCR biomaterial samples.

7.4. Transport and storage of the samples

Transport and storage conditions of biomaterial samples are stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples.

It is allowed to store biomaterial samples at 2 °C to 8 °C for no longer than 24 hours. If it is impossible to deliver samples into the lab within 24 hours, a one-time freezing is allowed. It is allowed to store frozen samples at temperature from minus 18 °C to minus 22 °C for one month (if it does not contradict the rules for NA extraction kits or transport media used for transport and storage of samples).

WARNING! Avoid repeated freezing and thawing of samples.

7.5. Sample preparation for DNA extraction

Peripheral blood, cerebrospinal fluid, amniotic fluid, scrapes from mucous membranes, feces, meconium

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

7.6. Biopsy specimens/punctate

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

7.7. If using PREP-NA and PREP-GS reagent kits for DNA extraction:

7.7.1 Vortex the tubes with samples for 3-5 seconds, then spin on vortex for 3-5 seconds.
7.7.2 Remove supernatant.

The sample is ready for DNA extraction.

8. PROCEDURE

DNA extraction from biological material

It is recommended to use DNA extraction reagent kits compatible with the relevant biomaterial for further DNA analysis by PCR, e.g. PREP-NA, PREP-GS, PREP-MB RAPID, PREP-RAPID, PREP-OPTIMA, PREP-MB MAX (see Table 5).

DNA extraction is carried out according to the instruction for use for the kit of reagents.

Table 5. Reagent kits validated for DNA extraction for further analysis using **Listeria monocytogenes REAL-TIME PCR Detection Kit**:

Reagent kit	Biomaterial	Minimum elution volume, µL
PREP-NA	Mucous scrapes, cerebrospinal fluid, biopsy specimens/punctate, feces, meconium	50
PREP-GS	Mucous scrapes, cerebrospinal fluid, biopsy specimens/punctate	100
PREP-MB RAPID	Mucous scrapes, cerebrospinal fluid, amniotic fluid	100
PREP-RAPID	Mucous scrapes, cerebrospinal fluid	500
PREP-OPTIMA	Whole peripheral blood	100
	Mucous scrapes, cerebrospinal fluid, amniotic fluid, biopsy specimens/punctate, feces, meconium	400
PREP-MB MAX	Whole peripheral blood, mucous scrapes, feces, meconium	50

WARNING!

1. Simultaneously with DNA extraction from biological material a negative control must be prepared and carried through all the stages of sample preparation. Physiological saline solution can be used as a negative control in volumes as indicated in the instructions for use of extraction kits or negative control that is included in the corresponding extraction kit.
2. Perform DNA extraction from biopsy specimens/punctate using PREP-NA and PREP-GS reagent kits according to **this instruction**.

8.1. DNA extraction from biopsy specimens/punctate using PREP-NA and PREP-GS reagent kits

8.1.1. General requirements

8.1.1.1. Use RNase and DNase free filter tips. Change the tips after each solution removal from the tube. When working with aspirator, use RNase and DNase free tips without filter.

8.1.1.2. When adding solution into the tube containing biomaterial, add the solution carefully without touching the walls of the tube. If touching occurs, change the tip.

8.1.1.3. To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube. It is prohibited to work with several tubes with open caps simultaneously.

8.1.1.4. Test samples and negative control (C-) must be treated in the same manner simultaneously according to this instruction.

8.1.2. DNA extraction from biopsy specimens/punctate using PREP-NA reagent kit

WARNING!

1. Before starting work, take the nucleic acid extraction kit out of the refrigerator and check if there is precipitate in lysis solution. If there is precipitate, heat the lysis solution at 65 °C until complete dissolution of the precipitate, then stir by turning the vial upside down 5-10 times. Avoid foaming.
2. Cap opening may occur during heating! Use tubes with locking caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp lid (e.g. solid-state thermostat TT-1-DNA-Tech manufactured by "DNA-Technology R&P", LLC).

8.1.2.1. Mark one 1.5 mL plastic tube for each test sample and for negative control (C-).

8.1.2.2. Add 300 µL of lysis solution into the tubes with prepared biopsy specimens/punctate samples and into the tube with C-. Avoid touching the walls of the tubes.

8.1.2.3. Add 100 µL of negative control into the C- tube. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.1.2.4. Heat the tubes on thermostat at 65 °C for 30 minutes and spin on vortex for 3-5 seconds.

8.1.2.5. Transfer supernatant into the corresponding marked tubes with test samples. Do not add supernatant into the C- tube.

8.1.2.6. Add 400 µL of precipitation reagent into each tube. Avoid touching the walls of the tubes. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.1.2.7. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 15 minutes.

8.1.2.8. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.1.2.9. Add 500 µL of wash solution No. 1 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and turn the tubes upside down carefully 3-5 times.

8.1.2.10. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 5 minutes.

8.1.2.11. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.1.2.12. Add 300 µL of wash solution No. 2 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and turn the tubes upside down carefully 3-5 times.

8.1.2.13. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 5 minutes.

8.1.2.14. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.1.2.15. Open the tubes and dry the precipitate at 65 °C for 5 minutes.

8.1.2.16. Add 50 µL of dilution buffer to the precipitate. Close the tubes.

8.1.2.17. Spin down the drops on vortex for 1-3 seconds.

8.1.2.18. Heat the tubes on thermostat at 65 °C for 10 minutes and shake on vortex for 3-5 seconds

8.1.2.19. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 30 seconds.

DNA preparation is ready for introduction into the PCR reaction mixture.

DNA preparation may be stored at temperature from minus 18 °C to minus 22 °C for up to one month and at temperature from minus 68 °C to minus 72 °C for up to one year.

Before using DNA preparation for PCR thaw the tubes with DNA preparation at room temperature (18 °C — 25 °C) or at temperature from 2 °C to 8 °C, shake on vortex for 3-5 seconds and spin for 1-3 seconds.

DNA preparation is ready for introduction into the PCR reaction mixture.

8.2. DNA extraction from biopsy specimens/punctate using PREP-GS reagent kit

8.2.1. Mark one 1.5 mL plastic tube for each test sample and for negative control (C-).

8.2.2. Add 150 µL of lysis solution into the tubes with prepared biopsy specimens/punctate samples and into the tube with C-. Avoid touching the walls of the tubes.

8.2.3. Add 50 µL of sterile physiological saline solution or transport medium into the C- tube. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.2.4. Heat the tubes on thermostat at 50 °C for 30 minutes and spin on vortex for 3-5 seconds.

8.2.5. Transfer supernatant into the corresponding marked tubes with test samples. Do not add supernatant into the C- tube.

8.2.6. Resuspend the sorbent thoroughly on vortex. Turn the tube upside down to make sure that the sorbent does not stick to the bottom of the tube.

8.2.7. Add 20 µL of previously resuspended sorbent into each tube (including C-). Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.8. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.9. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.2.10. Add 200 µL of wash solution No. 1 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.11. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.12. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.2.13. Add 200 µL of wash solution No. 2 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.14. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.15. Add 200 µL of wash solution No. 3 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.16. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.17. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.2.18. Open the tubes and dry the precipitate at 50 °C for 5 minutes.

8.2.19. Add 100 µL of elution solution to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 5-10 seconds.

8.2.20. Heat the tubes at 50 °C for 5 minutes.

8.2.21. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

Supernatant containing the extracted DNA is ready for introduction into the PCR reaction mixture

The obtained DNA preparation may be stored for up to 7 days at temperature from 2 °C to 8 °C. Before using DNA preparation for PCR repeat 8.2.20 — 8.2.21.

If you plan to store the sample for more than 7 days, transfer the supernatant to a separate tube and store it at temperature from minus 18 °C to minus 22 °C.

DNA preparation may be stored at temperature from minus 18 °C to minus 22 °C for up to 6 months.

Before using DNA preparation for PCR after storage, thaw the tubes with DNA preparation and negative control at room temperature (18 °C — 25 °C) or at temperature from 2 °C to 8 °C, shake on vortex for 3-5 seconds and spin for 1-3 seconds.

DNA preparation is ready for introduction into the PCR reaction mixture.

8.3. Preparing PCR for package S

WARNING!

1. The reagents and tubes should be kept away from direct sun light.
2. Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.3.1 Mark one tube/stripped tube with the paraffin sealed PCR-mix for each test sample, negative control (C-), positive control (C+).

WARNING! The volume of reagents is calculated for no more than 12 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

Example: To test 4 samples, mark 4 tubes for samples, one negative control tube "C-" and one positive control tube "C+". Total number of tubes is 6.

8.3.2 Shake the tubes with Taq-polymerase solution on vortex mixer for 3-5 seconds and spin in a vortex mixer for 1-3 seconds.

8.3.3 Add 10 µL of Taq-polymerase solution to each tube. Avoid paraffin layer break.

WARNING! If using Rotor-Gene Q detecting thermal cycler, do not add mineral oil!

8.3.4 Add one drop (~20 µL) of mineral oil into each strip tube. Close the tubes/strips.

8.3.5 Shake the tube with positive control on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR-mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using PREP-NA, PREP-RAPID and PREP-GS extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), mix the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using PREP-MB RAPID and PREP-MB MAX DNA extraction kit, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. If strips are used, close the strip caps after adding the sample before proceeding with the next sample. Close the tubes/strips tightly. Use filter tips.

8.3.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the tubes "C-", and "C+". Avoid paraffin layer break.

8.3.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube. Avoid paraffin layer break.

8.3.8 Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.3.9 Spin the tubes/strips for 3-5 seconds in a vortex mixer (if using Rotor-Gene Q, spinning is not necessary).

8.3.10 Set the strips into the real-time thermal cycler.

8.3.11 Launch the operating software for DT instrument[2]. Add corresponding test[3], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the strips in the thermal unit (see 8.9) and run PCR. See Table 6.

8.3.12 For Rotor-Gene Q, CFX96 and Applied Biosystems QuantStudio 5 detecting thermal cyclers: perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 7, 8, 9.

Table 6. The PCR program for DTlite and DTprime thermal cyclers

Step	**Temperature, °C**	**Min.**	**Sec.**	**Number of cycles**	**Optical measurement**	**Type of the step**
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	25[4]		…	Holding		Holding
√ - optical measurement						

Table 7. Amplification program for Rotor-Gene Q detecting thermal cycler (package S, tubes)

Cycling	Temperature, °C	Hold Time, s	Cycle Repeats
Cycling	80 deg	60	1 time
	94 deg	90	
Cycling 2	94 deg	30	5 times
	57 deg **√**	15	
Cycling 3	94 deg	10	45 times
	57 deg **√**	15	
√- optical measurements, set up fluorescence measurement (Acquiring) on Green (Fam) and Yellow (Hex) detection channels at 57 °C.			

Table 8. The PCR program for CFX96 thermal cyclers (packages S, U)

Step	Temperature, °C	Time min: sec	Number of cycles (repeats)
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 **√**	0:20	
√- optical measurements (Plate Read), set the fluorescence measurement on the Fam and Hex channels at 64 °C			

[2] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[3] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

[4] Holding at 10 °C is allowed

Table 9. The PCR program for Applied Biosystems QuantStudio 5 thermal cyclers (packages S, U)

Stage	Step	Temperature, °C	Time min: sec	Number of cycles (repeats)
Holding	1	80	01:00	1
	2	94	01:30	1
PCR	1	94	0:20	50
	2	64 **√**	0:20	
√- data collection for fluorophores (Fam, Vic (Hex)) is on				

8.4. Preparing PCR for package U, manual dosing

WARNING! For amplification use 0.2 mL single-use amplification tubes or 96-well PCR microplates[5], sealed hermetically with thermal film. It is not recommended to use strips due to increased risk of postamplification contamination.

WARNING! The reagents and tubes should be kept away from direct sunlight.

8.4.1 Mark the required number of 0.2 mL tubes or a 96-well microplate for each test sample, negative control (C-) and positive control (C+).

Note: It is recommended to test at least 5 samples per analysis (3 test samples, negative control and positive control).

Example: to test 4 samples, mark 4 tubes/reserve 4 wells for samples, 1 tube/well for "C-" and 1 tube/well for "C+". The resulting number of tubes/wells is 6.

8.4.2 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.4.3 Add 6.0 µL of PCR-mix to each tube/well (including "C-" and "C+").

8.4.4 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

WARNING! Take TechnoTaq MAX polymerase out from the freezer immediately prior to use.

8.4.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into the one tube:
6.0 x (N+1) µL of PCR-buffer,
0.3 x (N+1) µL of TechnoTaq MAX polymerase,
where N is the number of marked tubes/microplate wells considering "C-", "C+".

Example: to test 4 samples, "C-" and "C+" in one PCR run, prepare the mixture of PCR-buffer and Taq-polymerase for 7 (6+1) tubes/wells, i.e. 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.4.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin on vortex for 1-3 seconds to collect the drops.

WARNING! Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.4.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube/well with PCR-mix.

WARNING! Follow the steps 8.2.8 – 8.2.14 within two hours after adding PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.4.8 Vortex the tube with positive control "C+" for 3-5 seconds and spin down the drops for 1-3 seconds.

[5] - 96-well plates are not used with DTlite detecting thermal cycler

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR-mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using PREP-NA, PREP-RAPID and PREP-GS extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), mix the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using PREP-MB RAPID and PREP-MB MAX DNA extraction kit, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. Close the tubes/strips tightly. Use filter tips.

8.4.9 Add 6.0 µL of DNA sample into corresponding tubes/wells. Do not add DNA into the "C-", "C+" tubes/wells.

8.4.10 Add 6.0 µL of negative control ("C-") which passed whole DNA extraction procedure into the corresponding tube/well.

8.4.11 Add 6.0 µL of positive control sample ("C+") into the corresponding tube/well.

8.4.12 **In case of using 96-well microplates:**

8.2.12.1. Place the plate carefully, without shaking into the DTpack sealing device.

8.2.12.2. Seal the PCR microplate with polymer thermal film according to the DTpack operation manual.

8.2.12.3. Centrifuge the plate at RCF(g) 100 for 30 seconds.

8.4.13 **In case of using tubes:**
Spin the tubes for 3-5 seconds on vortex mixer (in case of using Rotor-Gene Q, spinning is not necessary).

8.4.14 Set the tubes into the real-time thermal cycler.

8.4.15 Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes in thermal unit (see 8.2.14) and run PCR. See Table 10.

8.4.16 For Rotor-Gene Q, CFX96 and Applied Biosystems QuantStudio 5 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Tables 8, 9, 11.

[6] Please, apply to Operation Manual for DTprime and DTlite real-time PCR instruments PART II.
[7] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 10. The PCR program for DTlite and DTprime thermal cyclers for package U

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
1	80	0	05	15		Cycle
	94	0	05			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	30*			Holding		Holding
√ - optical measurements * - holding at 10 °C is allowed						

Table 11. Amplification program for Rotor-Gene Q detecting thermal cycler (package U)

№ /Cycling	Temperature, °C	Hold Time, s	Cycle Repeats
Cycling	80 deg	60	1 time
	94 deg	300	
Cycling 2	94 deg	30	5 times
	57 deg **√**	15	
Cycling 3	94 deg	10	45 times
	57 deg **√**	15	
√- optical measurements, set up fluorescence measurement (Acquiring) on Green (Fam) and Yellow (Hex) detection channels at 57 °C.			

8.5. Preparing PCR for package U, using DTstream

WARNING! The reagents and tubes should be kept away from direct sun light.

WARNING! For amplification use 384-well microplates hermetically sealed with thermal film.

Note – It is recommended to test at least 5 samples in 1 run (3 test samples, negative control and positive control).

8.5.1 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.5.2 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

WARNING! TechnoTaq MAX polymerase should be taken out from the freezer immediately prior to use.

8.5.3 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase according to the software for DTstream.

8.5.4 Vortex the tube with the mixture PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.5.5 Vortex the tubes with positive control for 3-5 seconds and spin in vortex for 1-3 seconds to collect the drops.

WARNING!

1. Before dosing, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using PREP-NA, PREP-RAPID and PREP-GS extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), mix the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using PREP-MB RAPID and PREP-MB MAX DNA extraction kit, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

8.5.6 Set the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls and PCR microplate on the DTstream working table and conduct dosage of the components according to DTstream user manual.

8.5.7 After the end of dosing program on DTstream put the PCR microplate without shaking on the working table of DTpack sealing device.

8.5.8 Run the process of sealing of PCR microplate according to the user manual of DTpack sealing device.

8.5.9 Centrifuge the microplate at RCF(g) 100 for 30 seconds.

8.5.10 Set the PCR microplate into the real-time thermal cycler.

Launch the operating software for DT instrument[8]. Add corresponding test[9], specify the number and ID's of the samples, positive and negative controls. Specify position of the tubes in thermal unit and run PCR. The volume of reaction mixture is 18 µL. See Table 10.

9. CONTROLS

The **Listeria monocytogenes REAL-TIME PCR Detection Kit** contains positive control ("C+").

Positive control is a cloned part of the bacterial genome. It is produced with genetic engineering techniques and characterized by automatic sequencing.

TBL value is used for biomaterial sampling quality control and DNA extraction quality control.

To reveal possible contamination a negative control is required.

WARNING! A negative control should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

[8] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[9] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

10. DATA ANALYSIS

10.1. Registration of the results is carried out automatically during amplification by the software provided with detecting thermocycler.

10.2. When using CFX96 (Bio-Rad) detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the "Baseline Subtraction" tab select "Baseline Subtraction Curve Fit".

10.3. Result interpretation is carried out according to Table 12. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

Table 12. PCR results interpretation

Fam/Green, Cp/Cq/Ct	**Hex/Yellow/Vic**, Cp/Cq/Ct	**Result interpretation**
Specified	Not considered	***Listeria monocytogenes* DNA detected («+»)**
Not specified	**Specified**	*Listeria monocytogenes* not detected («-»)
Not specified	Not specified	Unreliable result
Not specified	**Specified**	**Negative result ("-")** Run results are valid
Specified	Not considered	**Positive result ("+")** Run results are valid

11. SPECIFICATIONS

a. Analytical specificity

In the human biomaterial samples containing DNA of *Listeria monocytogenes*, the PCR detector software ("PCR with endpoint fluorescence detection") or the thermal cycler software ("real-time PCR detection") registers positive amplification result for specific product (*Listeria monocytogenes* genome fragment) on Fam/Green detection channel.

In the human biomaterial samples not containing DNA of *Listeria monocytogenes*, the PCR detector software or the thermal cycler software registers negative amplification result for specific product (*Listeria monocytogenes* genome fragment) on Fam/Green detection channel and positive amplification result for internal control on Hex/Yellow/Vic detection channel.

Non-specific positive amplification results are absent if the following microorganisms are present in the DNA sample: *Listeria ivanovii*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Toxoplasma gondii*, Epstein Barr virus, Cytomegalovirus, *Lactobacillus* sp., and human DNA in concentration up to 1.0×10^8 copies/mL of sample.

b. Analytical sensitivity

5 copies of *Listeria monocytogenes* DNA per amplification tube.

Analytical sensitivity, or LOD (limit of detection) is determined by the analysis of serial dilutions of the laboratory controls (LC).

LOD depends on the used kit/set of reagents for DNA extraction and on the final volume of extracted DNA elution.

LOD of 5 DNA copies per amplification tube corresponds to the following DNA concentration values for nucleic acid extraction reagent kits manufactured by "DNA-Technology R&P", LLC and "DNA-Technology TS", LLC:

Biomaterial	DNA extraction reagent kit	Obtained preparation, µL	Limit of detection, copies/sample
Scrapes of epithelial cells in 500 µL of transport medium[10]	PREP-NA	50	50
	PREP-GS	100	100
	PREP-MB RAPID	100	100
	PREP-RAPID	500	500
	PREP-OPTIMA	400	400
	PREP-MB MAX	50	50
Cerebrospinal fluid (extraction from 500 µL of sample)	PREP-NA	50	50
	PREP-GS	100	100
	PREP-MB RAPID	100	100
	PREP-RAPID	500	500
Cerebrospinal fluid, amniotic fluid (extraction from 1.0 mL of sample)	PREP-OPTIMA	400	400
Amniotic fluid (extraction from 500 µL of sample)	PREP-MB RAPID	100	100
Biopsy specimens/punctate	PREP-NA	50	50
	PREP-GS	100	100
	PREP-OPTIMA	400	400
Feces, meconium in 1.0 mL of physiological saline solution	PREP-NA	50	50
	PREP-OPTIMA	400	400
	PREP-MB MAX	50	50
Whole peripheral blood (500 µL[11])	PREP-OPTIMA MAX	100	100
Whole peripheral blood (100 µL)	PREP-MB MAX	50	50

c. Diagnostic characteristics

Biomaterial	**Diagnostic sensitivity**	**Diagnostic specificity**
Amniotic liquid	100% (86.28-100)	100% (86.28-100)
Biopsy specimens or punctate from lesion of organs and tissues	100% (86.28-100)	100% (86.28-100)
Blood	100% (86.28-100)	100% (86.28-100)
Scrapes from mucous membranes	100% (86.28-100)	100% (86.28-100)
Cerebrospinal fluid	100% (86.28-100)	100% (86.28-100)
Feces or meconium	100% (86.28-100)	100% (86.28-100)
Total	**100% (97.57-100)**	**100% (97.57-100)**

d. Within-batch and between-batch precision

Within-batch precision – 100 % (92.60-100).

Between-batch precision – 100 % (92.60-100).

[10] - STOR-F transport medium manufactured by “DNA-Technology R&P”, LLC was used

[11] - when adding 100 µL of lysis solution

12. TROUBLESHOOTING

Table 13. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of **Listeria monocytogenes REAL-TIME PCR Detection Kit**.

Technical support:

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https://www.dna-technology.com

14. KEY TO SYMBOLS

	Temperature limit		Date of manufacture
	Contains sufficient for <n> tests		Consult instructions for use
	Use-by date		Catalogue number
	Batch code		Manufacturer
	Caution		Keep away from sunlight
	Non-sterile		Version
	For research use only		

R1-P003-S3/4EU
R1-P003-23/4EU
R1-P003-UA/9EU

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