IVD For *in Vitro* Diagnostic Use

CE

Listeria monocytogenes Real-TM Quant

Handbook

Real Time PCR Kit for quantitative detection of Listeria monocytogenes

REF B14-50FRT

Σ 50

REF B14-100FRT

Σ 100

NAME

Listeria monocytogenes Real-TM Quant

INTRODUCTION

Listeria is a genus of bacteria that contains ten species. Named after the English pioneer of sterile surgery Joseph Lister, the genus received its current name in 1940. Listeria species are Gram-positive bacilli. The major human pathogen in the Listeria genus is L. monocytogenes. It is usually the causative agent of the bacterial disease, listeriosis, a serious infection caused by eating food contaminated with the bacteria. The disease affects primarily pregnant women, newborns, adults with weakened immune systems, and the elderly.

INTENDED USE

Listeria monocytogenes Real-TM Quant kit is a Real-Time test for the Qualitative and Quantitative detection of *Listeria monocytogenes*.

PRINCIPLE OF ASSAY

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

Listeria monocytogenes DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel, the IC DNA amplification is detected on FAM (Green) channel, the signal of the β-globin gene (IC Glob) amplification product is detected on ROX(Orange) channel.

Quantitative DNA analysis is based on the linear dependence between the cycle threshold (Ct) and the initial concentration of DNA target. Quantitative analysis is performed in the presence of DNA calibrators (samples with a known concentration of *Listeria monocytogenes* DNA), which are added during amplification. The results of amplification of DNA calibrators are used to construct a calibration curve, on the basis of which the concentration of *Listeria monocytogenes* DNA in samples determined.

MATERIALS PROVIDED

Real Time PCR kit (B14-50FRT)

- **PCR-mix-1 *Listeria monocytogenes***, 0,6 ml
- **PCR-mix-2- FRT**, 0,3 ml
- **TE-buffer**, 0,2 ml
- **Negative Control C-***, 1,2 ml
- **Internal Control IC****, 0,6 ml
- **Pos C+ *Listeria monocytogenes****, 0,1 ml
- **Standard *Listeria* DNA**
  - **QS1**, 0,2 ml
  - **QS2**, 0,2 ml

Contains reagents for 55 tests.

Real Time PCR kit (B14-100FRT)

- **PCR-mix-1 *Listeria monocytogenes***, 2 x 0,6 ml
- **PCR-mix-2- FRT**, 2 x 0,3 ml
- **TE-buffer**, 2 x 0,2 ml
- **Negative Control C-***, 2 x 1,2 ml
- **Internal Control IC****, 2 x 0,6 ml
- **Pos C+ *Listeria monocytogenes****, 2 x 0,1 ml
- **Standard *Listeria* DNA**
  - **QS1**, 2 x 0,2 ml
  - **QS2**, 2 x 0,2 ml

Contains reagents for 110 tests.

** must be used during the sample preparation procedure: add 100 µl of C– (Negative Control C-) to the tube labeled Cneg;*

*** add 10 µl of Internal Control to all samples during the DNA isolation procedure directly to the sample/lysis mixture;*

**** must be used during sample preparation procedure: add 10 µl of Pos C+ Listeria monocytogenes and 90 µl of C– (Negative Control) to the tube labeled Cpos.*

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA isolation kit
- Desktop microcentrifuge for “eppendorf” type tubes
- Vortex mixer
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer
- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters

STORAGE INSTRUCTIONS

Listeria monocytogenes Real-TM Quant must be stored +2-8°C except for **PCR-mix-1** ***Listeria monocytogenes*** and **PCR-mix-2- FRT** that must be stored at -20°C. The **Listeria monocytogenes Real-TM Quant** kit can be shipped at 2-8°C but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY

Listeria monocytogenes Real-TM Quant Test is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

QUALITY CONTROL

In accordance with Sacace’s ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

IVD

***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification. Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

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

Sampling

The material for the analysis includes DNA samples extracted from:

- peripheral and umbilical cord blood;
- cerebro-spinal fluid;
- node aspirates;
- naso-pharyngeal swabs;
- the discharge of the eye conjunctiva;
- amniotic fluid;
- placenta;
- the scrapes of epithelial cells taken from vagina side parietes;
- urine;
- breast milk;
- meconium;
- feces;
- autopsy material;
- primary aliment fortification medium;
- the medium for primary fortification of environment objects (concentrated (eluated) water samples (discharged water, drinking water taken from land-based bodies of water, etc).

Pretreatment

- The pretreatment of peripheral and umbilical cord blood is carried out in the following way: add 1.0 ml of hemolytic (can be ordered separately) and 0.25 ml of blood into 1.5-ml “Eppendorf”-type tubes using separate tips. Gently mix the content of the tube using a vortex and leave it for 10 minutes stirring at times. Centrifuge the tubes using a microcentrifuge at 6000 g during 2 minutes. The supernatant liquid is to be taken with a vacuum aspirator leaving the deposit untouched. After washing the cells deposit (the leftovers of destroyed erythrocytes) should be white (only the pinkish coat above the deposit is acceptable). One can repeat the washing using hemolytic if necessary. The deposit of leucocytes is to be immediately lysed (in case of using “DNA/RNA-prep” extraction kit add 300 µl of lysis solution) or long-lastingly stored frozen at minus 68 °C and lower.
- Cerebro-spinal fluid samples need 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 by vortexing.

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 ≤ –68 °C – for a long time.

- Amniotic fluid is to be extracted into a sterile "Eppendorf"-type tube during the amniocentesis in accordance with the standard technique. It is required to carry out the pretreatment of the test material. Resuspend the amniotic fluid thoroughly. Take 1 ml of material with an adjustable pipette using a filter tip and pour it into a sterile "Eppendorf"-type tube for further centrifugation at 8-9.000 g during 10 minutes. After the process of centrifugation carefully take the supernatant fluid with a filter tip leaving 200 µl of fluid above the deposit. Then resuspend the material on the vortex. It is acceptable to store the amniotic fluid and pretreated material within 24 hours at 2-8 °C, within 1 month at minus 16 °C and lower. Long-term storage is allowed at minus 68 °C.
- Breast milk is to be pretreated before testing. Stir the breast milk sample by pipetting. Take 1 ml of material with an adjustable pipette using a filter tip and pour it into a sterile "Eppendorf"-type tube for further centrifugation at 8–9.000 during 5 minutes. Carefully take the supernatant leaving 100 µl of supernatant fluid, then resuspend the material on the vortex. It is acceptable to store the breast milk deposit within 24 hours at 2-8 °C, within 1 month at minus 16 °C and lower. Long-term storage is allowed at minus 68 °C.
- The urine samples need 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 resuspend salts, take only 1 ml of upper salt solution and then concentrate again. Completely remove the supernatant using vacuum aspirator with trap flask without disturbing the pellet. Add Transport medium with mucolytic agent at the finale volume of 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 ≤ –68 °C – for a long time.
- The samples of autopsy material and placenta need 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 ≤ –68 °C – for a long time.

- Concentrated (eluated) water samples are used for the extraction of *L.monocytogenes* DNA without any pretreatment. If there are any visible foreign substances or visible tinction in the test samples, the samples are to be stirred thoroughly on the vortex, then one should carry out the centrifugation during 1 minute at 10.000 g at room temperature. The supernatant fluid is used for the DNA extraction. The material is to be stored within 24 hours at 2–8 °C, within 1 month at minus 16 °C and lower. Long-term storage is allowed at minus 68 °C.

DNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the "SAMPLE COLLECTION, STORAGE AND TRANSPORT" paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

- ⇨ **DNA/RNA-Prep** (Sacace, REF K-2-9);
- ⇨ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006).

Please carry out DNA extraction according to the manufacture's instruction.

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

PROTOCOL:

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

1. Prepare required quantity of tubes or PCR plate.
2. Thaw the tubes with **PCR-mix-1** ***Listeria monocytogenes*** and **PCR-mix-2-FRT**. Thoroughly vortex the tubes and spin down the drops.
3. Prepare for each sample in the new sterile tube **10*N µl** of **PCR-mix-1** ***Listeria monocytogenes*** and **5*N µl** of **PCR-mix-2-FRT.**
4. Add **15 µl** of **Reaction Mix** into each tube.
5. Add **10 µl** of **extracted DNA** sample (including extracted controls) to the appropriate tube with Reaction Mix.
6. Prepare for qualitative analysis 1 positive control and 1 negative amplification control:
   - add **10 µl** of **QS2** to the tube labelled Amplification C*pos*;
   - add **10 µl** of **TE-buffer** to the tube labelled Amplification C*neg*;
7. Prepare for quantitative analysis 2 Standard QS1, 2 Standard QS2* and 1 negative amplification control:
   - add **10 µl** of **QS1** to each of the two tubes labelled Quantitative Standard 1;
   - add **10 µl** of **QS2** to each of the two tubes labelled Quantitative Standard 2;
   - add **10 µl** of **TE-buffer** to the tube labelled Amplification C*neg.*

   ** QS1 and QS2 values are specific for each lot and are reported in the Quant Data Card provided in the kit.*

Close tubes and transfer them into the instrument in this order: samples, negative controls, positive control, Standards. Create a temperature profile on your instrument* as follows:

Step	***Temperature, °C***	***Time***	***Fluorescent signal detection***	***Cycles***
1	50	15 min	–	1
2	95	15 min	–	1
3	95	10 s	–	45
	60	25 s	**FAM, JOE, ROX**	

** For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen), SaCycler-96™ (Sacace), CFX96™ /iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied Biosystems), SmartCycler® (Cepheid).*

INSTRUMENT SETTINGS

Settings for rotor-type instruments (Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q)

Channel	Calibrate / Gain Optimisation	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	from 5Fl to 10Fl	0,03	10 %	on
JOE/Yellow	from 5Fl to 10Fl	0,03	10 %	on
ROX/Orange	from 5Fl to 10Fl	0,03	10 %	on

Settings for plate-type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

RESULTS INTERPRETATION

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line. To set threshold put the line at such level where curves of fluorescence are linear.

- *L.monocytogenes* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel;
- IC glob (β-globin gene) DNA amplification is detected on ROX (Orange)/TexasRed channel (only for the total DNA extraction from cell suspension (whole blood, leucocytes, biopsy and autopsy material, swabs)
- Exogenous Internal Control IC is detected on FAM (Green) channel.

Qualitative analysis

The result of the qualitative analysis is considered reliable only if the results for Positive and Negative Controls of amplification as well as the Positive and Negative Control of extraction are in accordance with the table below and with the boundary values specified in the DataCard specific for each lot.

Results for controls (qualitative analysis):

Control	Stage for control	*Ct* value in the channel for fluorophore		
		FAM	JOE	ROX
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

Results interpretation for clinical samples (qualitative analysis):

***Ct* value in the channel for the fluorophore**			**Result**
FAM	**JOE**	**ROX**	
For whole blood, umbilical cord blood, placenta, swabs and scrapes, 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 eye conjunctiva, urine, meconium, feces, 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 result****

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

** In case of **equivocal result**, the PCR analysis should be repeated for the corresponding clinical sample starting from the DNA extraction stage. If in the new analysis it is still obtained the same result the sample is considered as positive. If in the new analysis it is obtained a negative result, the sample is considered as equivocal and a new sample collection is required.

Quantitative analysis

The result of the quantitative analysis is considered reliable only if the results for Positive and Negative Controls of amplification as well as the Positive and Negative Control of extraction and Quantitative Standards are in accordance with the table below and with the boundary values specified in the DataCard specific for each lot.

Results for controls (quantitative analysis):

Control	Stage for control	Result of amplification in the channel for fluorophore		
		FAM	JOE	ROX
PCE	DNA extraction	<boundary *Ct* value	<boundary *Ct* value; concentration value falls in the range	<boundary *Ct* value
C–	DNA extraction	<boundary *Ct* value	Absent	absent or > boundary *Ct* value
NCA	PCR	Absent	Absent	Absent
QS1	PCR	**Defined**	**Defined**	**Defined**
QS2	PCR	<boundary *Ct* value	<boundary *Ct* value	<boundary *Ct* value

Based on the Ct values obtained for each QS standard with known concentration (concentrations are specific for each lot as stated in the specific DataCard provided with the kit), is made a calibration curve against with are plotted the Ct values of the clinical samples giving the corresponding values of copies of L. monocytogenes DNA.

For the quantitative test the calculation of the DNA concentration of L. monocytogenes per 1 ml of clinical sample is carried out using the following formula:

*Copies of L.monocytogenes DNA/ml x Coefficient A= copies/ml**

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

** For example, with the extraction volume of 100 µl, the corresponding Coefficient A is 1. In this case is not even needed to apply the formula since the copies/ml value obtained through the Real Time software is equivalent to the final concentration of Listeria.*

Results interpretation for clinical samples (quantitative analysis):

Result	Interpretation
For whole blood, umbilical cord blood, placenta, swabs and scrapes, node aspirates and autopsy material	
Invalid	The *Ct* value for the FAM channel is absent or greater than the boundary *Ct* value, whereas the *Ct* value determined in the channel for 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 eye conjunctiva, urine, meconium, feces, 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 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**.

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of **Listeria monocytogenes Real-TM Quant** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The PCR kit detects *L.monocytogenes* DNA. The specific activity of the PCR kit is proved by testing different strains of *L.monocytogenes.* The absence of the kit components' activity is shown in reference of DNA of other causative agents such as: *Candida albicans*, *Chlamydophila pneumonia*, *Cryptococcus neoformans*, *Cytomegalovirus hominis*, *Epstein–Barr virus* (*EBV*), *Escherichia coli*, *Haemophilus haemolyticus*, *H.influenzae*, *H.parainfluenzae*, *Hepatitis A virus* (*HAV*), *Hepatitis B virus* (*HBV*), *Hepatitis C virus* (*HCV*), *Hepatitis D virus* (*HDV*), *Herpes simplex virus I* (*HSV I*), *virus II* (*HSV II*), *Human Herpes virus VI* (*HHV6*), *virus VII,* (*HHV7*), *Human adenovirus B*, *C*, *E*, *F*; *Human immunodeficiency virus* (*HIV*), *Human papillomavirus* 6, 11, 16, 18, 33, 35 (*HPV* 6, 11, 16, 18, 33, 35), *Klebsiella oxytoca*,

K.pneumonia, *Measles virus*, *Moraxella catarrhalis*, *Mumps virus*, *Mycobacterium tuberculosis*, *Mycoplasma pneumonia*, *N. cinereae*, *N.elongata*, *N.flavescens*, *N.gonorrhoeae*, *N.meningitidis*, *N.mucosa*, *N.sicca*, *N.subflava*, *Proteus mirabilis*, *P.vulgaris*, *Pseudomonas aeruginosa*, *Rubella virus*, *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *S.milleri*, *S.mitis*, *S.mutans*, *S.oralis,* *S.pneumoniae*, *S.pyogenes*, *S.salivarius*, *S.sanguis*, *S.suis*, *S.viridans*, *Toxoplasma gondii*, *Varicella–Zoster virus* and human DNA.

Analytical sensitivity

The kit **Listeria monocytogenes Real-TM Quant** allows to detect *Listeria monocytogenes* DNA in 100% of the tests with a sensitivity of not less than 500 copies/ml.

Linearity

Listeria monocytogenes Real-TM Quant is linear from 1 x 10^3 to 1 x 10^8 copies/ml.

QUALITY CONTROL PROCEDURE

A defined quantity of Internal Control (IC) is introduced into each sample and control at the beginning of sample preparation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

A negative control of extraction (NCE), negative amplification control (NCA), positive and negative amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

TROUBLESHOOTING

1. The *Ct* value obtained for the Positive Control of amplification (C+) in the FAM and/or JOE and/or ROX channels is greater than the boundary *Ct* value or absent: the amplification and detection should be repeated for all the samples.
2. The *Ct* value obtained for the Positive Control of Extraction (PCE) in the FAM and/or JOE and/or ROX channels is greater than the boundary *Ct* value specified in the DataCard or absent: the PCR analysis (starting from 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 fall in the range specified in the DataCard: the PCR analysis (starting from the DNA extraction stage) should be repeated for all samples.
4. If any *Ct* value is obtained for the Negative Control of Extraction (C–) in the JOE channel and if the *Ct* value in ROX channel is less than the boundary value: probably contamination of the laboratory with amplification fragments or reagents with consequent contamination at any stage of PCR analysis: take appropriate measures for detecting and elimination of contamination source. The PCR analysis (starting from the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
5. If any *Ct* value is obtained for the Negative Control of amplification (NCA) in any of the FAM and/or JOE and/or ROX channels: contamination of laboratory with amplification fragments, contamination of reagents or clinical samples is probable at any stage of PCR analysis. Take appropriate measures for detecting and elimination of contamination source. 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 QS calibrators in the specified detection channels. The amplification and detection should be repeated for all the samples.
7. For quantitative analysis, the *Ct* value is absent or greater than the boundary value for the QS2 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 setting and the concentrations of calibrators in accordance with the DataCard. If are still obtained improper results, amplification and detection for all the samples should be repeated.
9. If the *Ct* value is determined for the clinical sample, whereas the typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line): it is necessary to check the setting of the threshold line level or parameters of base line calculation. If the same result is still obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for the specific sample.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX™ and iQ5™ are registered trademarks of Bio-Rad Laboratories
* Rotor-Gene™ is a registered trademark of Qiagen
* MX3005P® is a registered trademark of Agilent Technologies
* ABI® is a registered trademark of Applied Biosystems
* SmartCycler® is a registered trademark of Cepheid

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