REF **B208-50FRT**

VER **02.03.10**

Legionella pneumophila/Legionella species Quant Real-TM

Real Time PCR kit for the detection of *Legionella species* and quantitative detection of *Legionella pneumophila* in the clinical materials (sputum, aspirate from trachea, nasopharyngeal swabs, throat swabs, bronchoalveolar lavage, tissue), microorganism cultures and in environmental samples (water, washes from environmental objects, biofilms scrapes, ground)

Σ **50**

NAME

Legionella pneumophila/Legionella species Quant Real-TM

INTRODUCTION

Legionella pneumophila is a thin, pleomorphic, flagellated Gram-negative bacterium of the genus *Legionella*. *L. pneumophila* is the primary human pathogenic bacterium in this group and is the causative agent of legionellosis or Legionnaires' disease.

Legionella pneumophila (named in memory of the deceased veterans) is ubiquitous to acquatic environments worldwide and resided as an intracellular parasite of amoeba and protozoa provided a link between natural environment and human disease. Thus, environmental monitoring, especially of potable water, cooling towers, and related sources, is a major focus in efforts to control the spread of this disease.

Since the initial identification of 235 cases in 1976 , Legionnaires disease has become recognized as the most common cause of atypical pneumonia in hospitalized patients. It is the second most common cause of community-acquired bacterial pneumonia with 25% mortality rate.

INTENDED USE

kit **Legionella pneumophila/Legionella species Quant Real-TM** is a test for Real Time qualitative detection of Legionella species and quantitative detection of Legionella pneumophila in the clinical materials (sputum, aspirate from trachea, nasopharyngeal swabs, throat swabs, bronchoalveolar lavage, tissue), microorganism cultures and in environmental samples (water, washes from environmental objects, biofilms scrapes, ground) with the possibility of differentiation of *Legionella pneumophila* from the other *Legionella species.*

There are more than 50 different types of Legionella but more than 90% of legionellosis cases are caused by *Legionella pneumophila*. Among other legionella species that can cause legionellosis there are *L. micdadei*, *L. longbeuchae*, *L. dumofii* and *L. bozemanii*: such species may cause disease only in presence of immunosuppression.

Sacace **Legionella pneumophila/Legionella species Quant Real-TM** kit allows not only to detect *Legionella spp* members but also to differentiate and quantify the most relevant type: *Legionella Pneumophila*.

PRINCIPLE OF ASSAY

kit **Legionella pneumophila/Legionella species Quant Real-TM** is a Real-Time Amplification test for the qualitative detection and differentiation of *Legionella pneumophila* in biological materials and in environmental samples.

Legionella pneumophila (mip gene) is detected on the Joe(Yellow)/Cy3/HEX channel, *Legionella species (16S rRNA)* on the Rox(Orange)/TexasRed channel and Positive inhibition Internal Control (Pos IC) on the Cy5(Red) channel (must be present in all samples).

MATERIALS PROVIDED

"Legionella pneumophila/Legionella species Quant Real-TM":

- PCR-mix-1, 2 x 0,6 ml;
- Taq Polymerase, 0,03 ml;
- Leg. Pneumophila/Leg.species/Pos IC C+, 0,1 ml;
- Negative Control C-, 0,1 ml;
- Standards:
  - QS1 Leg. Pneumophila, (10^7 copies), 0,1 ml;
  - QS2 Leg. Pneumophila (10^5 copies), 0,1 ml;
  - QS3 Leg. Pneumophila (10^3 copies), 0,1 ml

Contains reagents for 50 tests.

MATERIALS REQUIRED BUT NOT PROVIDED

- Real Time Thermalcycler
- Tubes or PCR plate
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks
- DNA extraction kit

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose all specimens and unused reagents in accordance with local regulations
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.

⚠ All pipetting steps have to be performed in a class II safety cabinet, since the samples are potentially infectious.

7. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
8. Material Safety Data Sheets (MSDS) are available on request.
9. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
10. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

Store kit **Legionella pneumophila/Legionella species Quant Real-TM** must be stored at -20°C. The kit can be shipped at 2-25°C but should be stored at -20°C immediately on receipt.

STABILITY

Legionella pneumophila/Legionella species Quant Real-TM 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. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

QUALITY CONTROL

The complete kit has been tested on an RotorGene 6000 (Corbett Research).
Certificates of Analyses are available on request at info@sacace.com.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Legionella pneumophila/Legionella species Quant Real-TM can analyze DNA extracted from:

- *Sputum, bronchial or tracheal lavage* must be treated with the following procedure*:
    - Collect sputum into 50 mL single-use PP tubes with a screw cap.
    - In a biological safety cabinet, homogenize samples after mixing with equal volume of 4% NaOH solution. (*N-acetyl-L-cysteine may be added if required in the amount of 50-70 mg per sample).* Mix intensely with a tube rotator for 5-20 minutes (depending on the density of a sample).
    - Centrifuge samples at 3000 rpm (2800-3000 g) for 15 min and carefully discard the supernatant leaving 500-1000 µl in the tube. Resuspend sediment and transfer it into a 1.5 ml tube.
    - Centrifuge samples at 12000 rpm for 5-10 min, discard the supernatant and use the same 1,5 ml sample tube for DNA isolation from sample sediment.
- *Nasopharyngeal and throat swabs:* insert the working area of the probe with cotton swab to sterile disposable tube with 500 µl of sterile saline or phosphate buffer solution (PBS). Broke off the terminal part of the probe or cut it off to allow dense closing of tube cup. Use the suspension for the DNA extraction.
- *Microorganism cultures,* suspected of *Legionella* spp: resuspend cultures in 0.5 ml of saline solution or phosphate buffer. Use 50 µl of suspension for DNA extraction
- *Tissue* (~1,0 gr) homogenized with mechanical homogenizer or scalpel, glass sticks, teflon pestles and dissolved in 1,0 ml of saline water or PBS sterile (1 volume of tissue to 1 volumes of saline solution). Vortex vigorously and incubate 30 min at room temperature. Transfer the supernatant into a new 1,5 ml tube;
- *Water (wastewater, from water reservoir, drinking water)*: 0.5 L of water is preliminary filtered through paper filter with glass funnel. After preliminary filtration water is filtered through membrane filter with pore diameter not more than 0.45 µm. After filtration membrane filter is chopped by sterile scissors (to disposable Petri dish) and placed by sterile pincers to 1.5 ml tubes with 1 ml of saline solution. The tube is incubated at room temperature during 15-20 min, periodically mixing on vortex for ensuring of microflora transition in solution. Use 50 µl of solution for DNA extraction.
- *Washes from environmental objects* are obtained by probe with cotton swab, saturated in sterile saline solution. Working end of probe with swab is placed in tube with 1.5 ml of saline solution, another part of probe is broken off and moved away. Use 50 µl of solution for DNA extraction.
- *Biofilms scrapes from internal surface of water supply, industrial and other equipment* (for example, from tray inside air-conditioners). Scrapes of moist biofilms under water or on the water-air interface are obtained by dry

sterile probe and scrapes of dried biofilms are obtained by swab, saturated in sterile saline solution. Working end of probe with swab is placed in 1.5 ml tube with 0.5 ml of saline solution, another part of probe is broken off and moved away. Use 50 µl of solution for DNA extraction.

- Ground (100 g): transfer the ground (0.4-1.0 g) to the tubes of 5 ml with tightly closable lid. Add 3 ml of saline solution in each tube, mix careful and decant 5 min. Supernatant (50 µl) is used for DNA extraction.

Specimens can be stored at +2-8°C for no longer than 48 hours, or freeze at -20°C to -80°C.
Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

PROTOCOL:

1. Prepare required quantity of reaction tubes for samples (N) and controls: N+2 for qualitative assay or N+4 for quantitative assay .
2. Prepare in the new sterile tube for each sample **20*N µl** of **PCR-mix-1** and **0,5*N** of **Taq DNA Polymerase.** Vortex and centrifuge for 2-3 sec.
3. Add **20 µl** of **Reaction Mix** and **5 µl** of **extracted DNA** sample to appropriate tube.
4. For each qualitative test prepare the following controls:
   - add **5 µl** of **Neg Control** to the tube labeled Amplification Negative Control;
   - add **5 µl** of **Pos C+** to the tube labeled Amplification Positive Control;
5. For each quantitative *Legionella pneumophila* test prepare the following controls:
   - add **5 µl** of **Neg Control** to the tube labeled Amplification Negative Control;
   - add **5 µl** of **QS1** to the tube labeled QS1;
   - add **5 µl** of **QS2** to the tube labeled QS2;
   - add **5 µl** of **QS3** to the tube labeled QS3.
6. Insert the tubes in the thermalcycler.

Table.1 Temperature profile

	Rotor type instruments[1]				*Plate type or modular instruments*[2]			
Stage	*Temp, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*	*Temp,°C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	95	300s	–	1	95	5 min	–	1
	62	50 s	JOE(Yellow), ROX (Orange), Cy5(Red)	50	62	60 s	JOE/HEX/Cy3, ROX/TexasRed, Cy5(Red)	50
	95	20 s	–		95	20 s	–	

[1] For example Rotor-Gene™ 3000/6000 (Corbett Research, Australia)
[2] For example, iQ5™/iQ iCycler™ (BioRad, USA); Mx3000P/Mx3005P™ (Stratagene, USA), Applied Biosystems® 7300/7500 Real Time PCR (Applera), SmartCycler® (Cepheid)

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line.
Legionella pneumophila is detected on the Joe(Yellow)/Cy3/HEX channel, *Legionella species* on the Rox(Orange)/TexasRed channel and Positive inhibition Internal Control (Pos IC) on the Cy5(Red) channel.