Sacace BIOTECHNOLOGIES

IVD For *in Vitro* Diagnostic Use

CE

MRSA Quant Real-TM

Handbook

Real Time PCR test for quantitative detection of methicillin resistant Staphylococcus aureus (MRSA) DNA

REF **B78-100FRT**

Σ **100**

NAME

MRSA Quant Real-TM

INTRODUCTION

S. aureus is a major cause of nosocomial infections. Most transmissions occur through the contaminated hands of a person carrying S. aureus. Treatment of S. aureus infections has become a real challenge with the emergence of strains resistant to previously effective antimicrobial agents.

Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is called MRSA any strain of Staphylococcus aureus that has developed resistance to beta-lactam antibiotics, including penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and cephalosporins. A defining characteristic of MRSA is its ability to thrive in the presence of penicillin-like antibiotics, which normally prevent bacterial growth by inhibiting synthesis of cell-wall material. MRSA contains a gene, mecA, which stops β-lactam antibiotics from inactivating the enzymes (transpeptidases) that are critical to cell wall synthesis.

In most patients, MRSA can be detected by swabbing the nostrils and isolating the bacteria found inside. Combined with extra sanitary measures for those in contact with infected patients, screening patients admitted to hospitals has been found to be effective in minimizing the spread of MRSA in hospitals in the United States, Denmark, Finland, Netherlands and other countries.

MRSA may progress substantially within 24–48 hours of initial topical symptoms. After 72 hours, MRSA can take hold in human tissues and eventually become resistant to treatment. The initial presentation of MRSA is small red bumps that resemble pimples, spider bites, or boils; they may be accompanied by fever and, occasionally, rashes. Within a few days, the bumps become larger and more painful; they eventually open into deep, pus-filled boils. About 75 percent of community-associated (CA-) MRSA infections are localized to skin and soft tissue and usually can be treated effectively. But some CA-MRSA strains display enhanced virulence, spreading more rapidly and causing illness much more severe than traditional healthcare-associated (HA-) MRSA infections, and they can affect vital organs and lead to widespread infection (sepsis), toxic shock syndrome, and necrotizing ("flesh-eating") pneumonia.

MRSA infections are associated with high mortality, morbidity, and costs. Thus, preventing MRSA acquisition is a top priority at many hospitals. One preventive approach is screening at admission for MRSA using rapid PCR testing, which can detect MRSA within 2 hours, as opposed to the 2, 3 up to 4 days necessary for conventional cultures. Using PCR testing for MRSA screening of hospitalized patients provides results more quickly and can lead to appropriate prevention measures.

INTENDED USE

MRSA Quant Real-TM PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantification of methicillin resistant *Staphylococcus aureus* (MRSA) DNA in the clinical materials (sputum, oropharyngeal swabs, urine sediment, bronchoalveolar lavage, plasma, tissue, flushing of medical instruments) by using real-time hybridization-fluorescence detection.

PRINCIPLE OF ASSAY

MRSA determination by the polymerase chain reaction (PCR) with hybridization fluorescent detection includes three stages: DNA extraction from clinical samples, PCR-amplification of pathogen genome specific region, and real-time hybridization fluorescent detection. DNA is extracted from sputum, oropharyngeal swabs, urine sediment, bronchoalveolar lavage, plasma, tissue, flushing of medical instruments in presence of Internal Control (IC), which allows to monitor the analysis of each sample. Then, *MRSA* DNA is amplified using specific primers and polymerase. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of the fluorescence intensities during the real-time PCR allows the detection of PCR product without re-opening the reaction tubes after the PCR run.

MRSA Quant Real-TM PCR kit detect the DNA fragment of *Staphylococcus aureus* and the DNA fragment of mecA gene specific only for methicillin resistant strains of *S.aureus*.

Staphylococcus aureus DNA is detected in the FAM/Green channel, MRSA DNA (ampliification of mecA fragment) is detected in the JOE/HEX/Yellow channel, Internal Control (IC) DNA is detected in the ROX/Orange channel.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (B78-100FRT)

- **PCR-mix-1-FRT *MRSA,*** 1.2 ml
- **PCR-mix-2-FRT,** 0.6 ml
- **TaqF Polymerase,** 0.06 ml
- **DNA-buffer,** 0.5 ml
- **DNA standard QS1 *MRSA,*** 0.2 ml
- **DNA standard QS2 *MRSA,*** 0.2 ml
- **Negative Control (C–)***, 2 x 1.2 ml
- **Positive Control DNA *MRSA***,** 0.1 ml
- **Internal Control (IC)***, 2 x 0.6 ml

Contains reagents for 100 tests

* *must be used in the extraction procedure as Negative Control of Extraction.*
** *must be used in the extraction procedure as Positive Control of Extraction (PCE).*
*** *add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see DNA/RNA-prep* ***REF*** *K-2-9).*

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Automatic adjustable pipettes (from 5 to 20 µl and from 20 to 200 µl).
- Disposable tips with aerosol barriers (100 or 200 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Real Time PCR instrument.
- Disposable polypropylene microtubes for PCR or PCR-plate
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

STORAGE INSTRUCTIONS

All components of the **MRSA Quant Real-TM** PCR kit (except for **PCR-mix-1-FRT *MRSA***, **PCR-mix-2-FRT**, and **TaqF Polymerase**) are to be stored at 2–8 ºC when not in use. All components of the **MRSA Quant Real-TM** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT *MRSA*, **PCR-mix-2-FRT** and **TaqF Polymerase** are to be stored at ≤ –16ºC.

PCR-mix-1-FRT *MRSA* is to be kept away from light.

STABILITY

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

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.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

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 (UNI EN ISO 18113-2:2012). 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 AND TRANSPORT

MRSA Quant Real-TM PCR kit is intended for the analysis of DNA extracted by using DNA extraction kits from sputum, oropharyngeal swabs, urine sediment, bronchoalveolar lavage, plasma, tissue, flushing of medical instruments.

Plasma

- Whole blood collected in EDTA should be separated into plasma and cellular components by centrifugation at 800-1600 x g for 20 min within six hours. The isolated plasma has to be transferred into a sterile polypropylene tube. Plasma may be stored at 2-8°C for an additional 3 days. Alternatively, plasma may be stored at -18°C for up to one month or 1 year when stored at -70°C.
- Do not freeze whole blood.
- Specimens anti-coagulated with heparin are unsuitable for this test.
- Thaw frozen specimens at room temperature before using.
- Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma may be transported at 2-8°C or frozen.

Sputum:

- 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 according to 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.

Bronchial lavage:

- centrifuge 10 mL at 7000 g/min for 10-15 min. Remove and discard the supernatant. If the pellet is not visible add 10 ml of liquid and repeat centrifugation, remove and discard the supernatant. Resuspend the pellet in 100 µl of saline water.

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;

Swabs:

- insert the swab into the nuclease-free 1,5 ml tube and add 0,2 mL of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

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);
- ⇨ **DNA-Sorb-B** (Sacace, REF K-1-1/B);
- ⇨ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006) for samples like sputum, oropharyngeal swabs, urine sediment, bronchoalveolar lavage, plasma

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

DNA is extracted from each clinical sample in the presence of **Internal Control** (**10 µl** of **IC** is added to each sample).
Transfer **100 µl** of **Negative Control** to the tube labeled C–.
Transfer **90 µl** of **Negative Control** and **10 µl** of **Positive Control DNA *MRSA*** to the tube labeled PCE.

PCR PROTOCOL

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

1. Prepare the mixture of **PCR-mix-2-FRT** and **TaqF polymerase**. For this purpose transfer the content of the tube with **TaqF polymerase (60 µl)** into the tube with **PCR-mix-2-FRT (600 µl)** and mix by vortexing without foam forming. Mark the date of mixture preparation.

The prepared mixture is intended for 120 samples analysis. Mixture is to be stored at the temperature between 2 ºC and 8 ºC for 3 months. Use when needed.

If you don't need to use the whole volume in 3 months, it is possible to prepare a lower quantity of Mix of PCR-mix-2-FRT and TaqF Polymerase at the rate of 10 to 1 (for example **150 µl** of **PCR-mix-2-FRT** and **15 µl** of **TaqF Polymerase).**

2. Prepare the reaction mixture. Note that for analysis of even one clinical DNA sample in the quantitative format, it is necessary to run five controls of PCR amplification stage: two standards (**QS1 *MRSA* and QS2 *MRSA***) in two replicates and the negative control of amplification (DNA-buffer). In addition, you should take reagents for one extra reaction.
3. Prepare required quantity of reaction tubes for samples and controls and add for each tube **10 µl** of **PCR-mix-1-FRT *MRSA*** and **5 µl** of **Mix** (PCR-mix-2-FRT with TaqF Polymerase).
   Calculate the required number of reactions with allowance for the clinical and control samples. See Table 1.

If 60 samples are analyzed simultaneously, you can use a simplified version of mixture preparation: transfer the content of one tube with PCR-mix-2-FRT and the content of one tube with TaqF polymerase to the tube with PCR-mix-1-FRT *MRSA*

4. Take the required quantity of tubes for amplification of clinical and control DNA samples. Transfer **15 µl** of the prepared mixture to each tube.
5. Add **10 µl** of **DNA** obtained from clinical or control samples to the tubes with the reaction mixture.
6. Prepare control amplification reaction:

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

Standards:

QS1 *MRSA* - Add **10 µl** of **QS1 *MRSA*** to two tubes and **10 µl** of **QS2 *MRSA*** to other two tubes.

QS2 *MRSA*

Table 1.

REACTION MIXTURE PREPARATION

	Reaction volume	
Reagent volume for 1 reaction (µl)	**10,0**	**5,0**
Number of clinical samples	**PCR-mix-1-FRT *MRSA****	**Mixture of PCR-mix-2-FRT and TaqF polymerase ***
1	**70**	**35**
2	**80**	**40**
3	**90**	**45**
4	**100**	**50**
5	**110**	**55**
6	**120**	**60**
7	**130**	**65**
8	**140**	**70**
9	**150**	**75**
10	**160**	**80**
11	**170**	**85**
12	**180**	**90**
13	**190**	**95**
14	**200**	**100**
15	**210**	**105**
16	**220**	**110**

*Values are given with account of one Negative Control of Extraction (NCE) and five PCR controls (2 Standards QS1 MRSA and QS2 MRSA (in two replicates) for quantitative analysis of *MRSA* DNA and negative control (DNA-buffer).

Amplification

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

	Rotor-type Instruments[1]			Plate- or modular type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	15 s	5	**95**	15 s	5
	60	30 s		**55**	30 s	
	72	15 s		**72**	15 s	
3	**95**	15 s	40	**95**	15 s	40
	55	30 s *fluorescent signal detection*		**55**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

[1] *For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)*
[2] *For example, SaCycler-96™ (Sacace), CFX-96™/ iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied), SmartCycler® (Cepheid), LineGeneK® (Bioer)*

INSTRUMENT SETTINGS

Rotor-type instruments (RotorGene 3000/6000, RotorGene Q)

Channel	Threshold	More Settings/ Outlier Removal	Calibrate / Gain Optimisation…	Slope Correct
FAM/Green	*0.03*	*10 %*	from 5Fl to 10Fl	*On*
JOE/Yellow	*0.03*	*10 %*	from 5Fl to 10Fl	*On*
Rox/Orange	*0.03*	*10 %*	from 5Fl to 10Fl	*On*

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.

DATA ANALYSIS

Staphylococcus aureus DNA is detected in the FAM/Green channel, *MRSA* DNA (ampliification of *mecA* fragment*)* is detected in the JOE/HEX/Yellow channel, Internal Control (IC) DNA is detected in the ROX/Orange channel.

Interpretation of results

The results are interpreted by the software of the PCR instrument used by the crossing (or not crossing) of the fluorescence curve with the threshold line. Principle of interpretation is presented in Table 3

Table 2. Results interpretation and quantitative analysis

Channel			Result (copies/ml)
FAM (*S.aureus*)	**JOE (mec A gene)**	**ROX (IC)**	
+	**-**	**+/-**	***MSSA* (= (A/C)*IC coefficient*N)**
-	**+**	**+/-**	***MRCoNS* (= (B/C)* IC coefficient*N)**
+	**+**	**+/-**	**Calculate lg A e B.** **1)** If the difference between **A** and **B** is not more than **0,3 lg,** result is ***MRSA* (= (B/C)* IC coefficient*N)** **2)** If **A>B** more than **0,3 lg,** result is ***S.aureus* (= (A/C)* IC coefficient *N),** including ***MRSA*** or ***MRCoNS* (= (B/C)* IC coefficient *N)** **3)** If **A<B** more than **0,3 lg,** result is ***MRCoNS*** or ***MRCoNS* and *MRSA* (= (B/C)* IC coefficient *N)**
-	**-**	**-**	**Invalid**
-	**-**	**+**	**Not detected**

A – concentration in FAM/Green channel

B – concentration in JOE/HEX/Yellow channel

C – concentration in ROX/Orange channel

N=100 / extraction volume, ml

IC coefficient is specific for each lot and reported in the Quant Data Card provided in the kit.

1. ***MSSA* (methicillin-sensitive *Staphylococcus aureus*)** DNA is detected if Ct value in the FAM/Green channel is defined and there is no Ct values for JOE/HEX/Yellow channel (fluorescence curve does not cross the threshold line).

Concentration is calculated using the following formula:

(A/C)*IC coefficient*N = (copies/ml)

A – concentration in FAM/Green channel

C – concentration in ROX/Orange channel

N= 100 / extraction volume, ml

2. ***MRCoNS* DNA (methicillin-resistant coagulase-negative *Staphylococcus aureus*)** DNA is detected if Ct value in the JOE/HEX/Yellow channel is defined and there is no Ct values for FAM/Green channel (fluorescence curve does not cross the threshold line).

Concentration is calculated using the following formula:

(B/C)*IC coefficient*N = (copies/ml)

B – concentration in JOE/HEX/Yellow channel

C – concentration in ROX/Orange channel

N= 100 / extraction volume, ml

3. ***MRSA* (methicillin-resistant *Staphylococcus aureus*)** DNA is detected if Ct value is defined in FAM/Green and JOE/HEX/Yellow channels and the difference between calculated concentration in FAM/Green and JOE/HEX/Yellow channels is not more than 0.3 lg.

Concentration is calculated using the following formula:

(B/C)*IC coefficient*N = (copies/ml)

B – concentration in JOE/HEX/Yellow channel

C – concentration in ROX/Orange channel

N=100 / extraction volume, ml

4. If calculated concentration in FAM/Green is more than concentration in JOE/HEX/Yellow channels and the difference is more than 0.3 lg, the result is ***Staphylococcus aureus,* including *MRSA* or *MRCoNS***.

Concentration of *Staphylococcus aureus* is calculated using the following formula:

(A/C)*IC coefficient*N = (copies/ml)

A – concentration in FAM/Green channel

C – concentration in ROX/Orange channel

N=100 / extraction volume, ml

Concentration of *MRSA* or *MRCoNS DNA* is calculated using the following formula:

(B/C)*IC coefficient*N = (copies/ml)

B – concentration in JOE/HEX/Yellow channel

C – concentration in ROX/Orange channel

N=100 / extraction volume, ml

5. If calculated concentration in FAM/Green is less than the concentration in JOE/HEX/Yellow channels and the difference is more than 0.3 lg, the result is ***MRCoNS* or *MRCoNS* and *MRSA*.**

Concentration is calculated using the following formula:

(B/C)*IC coefficient*N = (copies/ml)

B – concentration in JOE/HEX/Yellow channel

C – concentration in ROX/Orange channel

N=100 / extraction volume, ml

- Result is considered to be negative if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the FAM/Green and JOE/HEX/Yellow channels and the Ct value in the results grid in the ROX/Orange channel does not exceed the Ct value indicated in the **Data Card**.
- Result is considered to be **invalid** if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the FAM/Green and JOE/HEX/Yellow channels and the in the ROX/Orange Ct value is absent or exceeds the Ct value specified in the **Data Card**. Analysis should be repeated again.

Concentration of standards are specified in Data Card.

Internal control coefficient is specified in Data Card provided with each lot of the reagent kit. This coefficient is specific for each lot and can't be used for results calculation of PCR-kit of other lots.

Results of analysis are accepted as relevant if the results obtained for positive and negative controls of amplification and the negative control of extraction are correct. For quantitative analysis, the results for C+ should enter into the concentration range indicated in the **Data Card.**

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 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.

Results for controls

Results of analysis are accepted as relevant if the results obtained for positive and negative controls of amplification and the negative control of extraction are correct (table 3).

Table 3. Results for controls

Control	Stage for control	Ct in channel		
		FAM/Green	JOE/HEX/Yellow	*Orange*/Rox
NCE	DNA extraction	Neg	Neg	Pos (<boundary value)
PCE	DNA extraction, Amplification	Pos (< boundary value)	Pos (< boundary value)	Pos (< boundary value)
NCA	Amplification	Neg	Neg	Neg
QS1*MRSA*, QS2 *MRSA*	Amplification	Ct value and calculated concentration are determined	Ct value and calculated concentration are determined	Ct value and calculated concentration are determined

SPECIFICATIONS

Sensitivity

Linear range of **MRSA Quant Real-TM** PCR kit is **800–10.000.000 copies/ml**. If the result is greater than 10.000.000 copies/ml, it is indicated as ***the result is more than 10.000.000 MRSA DNA copies/ml***. If the result is less than 800 copies/ml, it is indicated as ***the result is less than 800 MRSA DNA copies/ml***.

The analytical sensitivity of **MRSA Quant Real-TM** PCR kit is given in the table 4.

Table 4. The analytical sensitivity

Type of clinical material	Nucleic acid extraction kit	Sensitivity
Sputum, oropharyngeal swabs, urine sediment, bronchoalveolar lavage, plasma, tissue, flushing of medical instruments	DNA/RNA-prep	400 copies/ml

Specificity

Specific activity of **MRSA Quant Real-TM** PCR kit was confirmed in studies of bacterial strains of *Staphylococcus aureus* including *MRSA*, as well as by analyzing clinical material with subsequent confirmation of results by sequencing the amplification fragments. Specificity of the kit was checked against strains of *Listeria monocytogenes, Moraxella catarrhalis, Pseudomonas aeruginosa, Proteus mirabilis*, *Neisseria meningitidis*, *N.cinereae, N.elongata, N.flavescens, N.gonorrhoeae, N.mucosa, N.sicca, N.subflava, Streptococcus pneumoniae, S.agalactiae, S.milleri, S.mitis, S.mutans, S.pyogenes, S.salivarius, S.sanguis, S.suis, S.viridans, Haemophilus influenzae, H.parainfluenzae, H.haemolyticus, Escherichia coli, Klebsiella pneumonia* and *K.oxytoca*. The clinical specificity of **MRSA Quant Real-TM** PCR kit was confirmed in laboratory clinical trials showing a value of 100%.

TROUBLESHOOTING

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

1. If invalid result has been obtained it is necessary to repeat PCR of the specific sample
2. Absence of positive signal in calibrators can result from incorrect settings of amplification program or failures of PCR pipetting. In that case it is necessary to repeat PCR again for all samples.
3. If there is a signal in Negative control of Extraction (C-) in FAM/Green and/or JOE/HEX/Yellow channels and in Negative control of amplification (NCA) in FAM/Green, JOE/HEX/Yellow and/or ROX/Orange channels, it means that contamination of reagents or samples has occurred. In that case results for all samples are considered to be invalid. Repeated PCR is required as well as countermeasures to prevent contamination;
4. If fluorescent curve does not have exponential slope (it more looks like a straight line) it means that threshold or baseline parameters have been set incorrectly. This result can't be considered as positive. If threshold value was correct it is necessary to repeat PCR for this sample.

KEY TO SYMBOLS USED

REF	List Number
LOT	Lot Number
IVD	For *in Vitro* Diagnostic Use
	Store at
	Manufacturer
	Consult instructions for use
	Expiration Date
QS1 MRSA **QS2 MRSA**	DNA Standards

	Caution!
	Contains sufficient for <n> tests
VER	Version
NCA	Negative Control of Amplification
NCE	Negative control of Extraction
C+	Positive Control of Amplification
IC	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX-96™, iQ5™ are a registered trademarks of Bio-Rad Laboratories
* Rotor-Gene™ Technology is a registered trademark of Qiagen
* MX3005P® is a registered trademark of Agilent Technologies
* ABI® is a registered trademark of Applied Biosystems
* LineGeneK® is a registered trademark of Bioer
* SmartCycler® is a registered trademark of Cepheid

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314892926
mail: ***info@sacace.com*** *web:* ***www.sacace.com***