RIDA®GENE MRSA
real-time PCR

Art. No.: PG0605
100 Reactions

For *in-vitro* diagnostic use.

-20 °C

R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany
Tel.: +49 (0) 61 51 81 02-0 / Telefax: +49 (0) 61 51 81 02-20

1. Intended use

For *in vitro* diagnostic use. RIDA®GENE MRSA is a multiplex real-time PCR for the direct, qualitative detection and differentiation of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) or methicillin-resistant coagulase-negative *Staphylococci* from human nasal or nasal/throat swabs, cultures, wound, axilla, groin and perineum swabs.

RIDA®GENE MRSA real-time PCR is intended for use as an aid in diagnosis of MRSA infection.

2. Summary and explanation of the test

Staphylococci commonly colonise the skin and mucous membranes of humans and other mammals. They are classified into coagulase-positive (*S.aureus*) and coagulase-negative *staphylococci* (e.g. *S. epidermidis*).
Staphylococcus aureus is a major cause of nosocomial infections in hospitals and healthcare settings.[1,2] Transmission occurs through health care providers or other patients. It is estimated that 30% of the healthy population are colonised (asymptomatic carriers) with *S. aureus.* Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of nosocomial infections worldwide (hospital-acquired MRSA also called HA-MRSA). Beside HA-MRSA infections also community-acquired MRSA infections (CA-MRSA) occur, which are acquired outside the hospital.[3,4] In the recent years also MRSA infections associated with livestock (livestock-associated MRSA or LA-MRSA) emerged, especially with pig farmers.[5,6]
MRSA are methicillin (oxacillin) resistant by virtue of the chromosomal mecA gene that encodes the penicillin binding protein (PBP2a). The mecA gene is located on a mobile genetic element called SCCmec gene cassette (Staphylococcal cassette chromosome mec). Today, eleven SCCmec types are described, of which types I to V are the most common.[3,7]
The SCCmec element type XI, which contains a new mecA homologue (mecC or mec_{LGA251}) was initially described in 2011. The mecC gene exhibits only a 70% nucleotide homology with mecA and is not detectable by usual mecA-specific PCRs and PBP2a agglutinations tests. This has been described in *S. aureus* isolates from humans and cattle.[8]
In contrast to infections with MSSA (Methicillin-sensitive *Staphylococcus aureus*), MRSA infections are associated with a higher morbidity, mortality, long lasting hospitalization and higher health care costs.[9,10] Risk factors for MRSA infection within healthcare settings include prolonged hospital stay, history of MRSA infection or colonisation, proximity to patients infected with MRSA and prolonged antibiotic treatment.[11] Every MRSA infection causes up to $ 10.000 additional costs.[12] In the

European Union more than 150.000 hospital patients get infected with MRSA each year. The associated health care costs for the European health care system are estimated to be 380 million Euro.[13] An early, fast and systematic MRSA screening enables a specific treatment of infected patients and an introduction of appropriate hygiene interventions prevents a MRSA-transmission and spread. Conventional culture-based methods for detection of MRSA require 48 to 72 hours. Real-time PCR assays enable an early and rapid MRSA screening on the day of hospital admission as part of an infection prevention program ("search and destroy" strategy).[14]

3. Test principle

The RIDA®GENE MRSA is a multiplex real-time PCR for the direct, qualitative detection and differentiation of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) or methicillin-resistant coagulase-negative *Staphylococci.*

After DNA-isolation, amplification of the mecA / mecC gene, SCCmec / orfX junction (types I, II, III, IV,V, VI, VII, IX, X and XI) and the SA442 gene (if present) specific for MRSA occurs.

The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE MRSA assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and to determine possible PCR-inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount	Lid Color
1	Reaction Mix	2x 1100 µl	yellow
2	Taq-Polymerase	1x 11 µl	red
D	Internal Control DNA	2x 1800 µl	orange
N	PCR Water	1x 500 µl	white
P	Positive Control	1x 200 µl	blue
L	Lysis Buffer 1	2x 12 ml	colorless

5. Storage instructions

- Protect all reagents, other than the Lysis Buffer 1, from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Lysis Buffer 1 can also be stored at 2 – 8 °C and can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw and fully defrost reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Lysis Buffer 1 should be fully defrosted and returned to room temperature before use.
- Reagents can sustain up to 5 freeze/thaw cycles without influencing the assay performance. (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all reagents, other than the Lysis Buffer 1, should be stored cold in an appropriate way (2 - 8 °C).

6. Additional equipment and materials required

- Sterile, media-free Rayon or Nylon flocked swabs (e.g. Copan Diagnostic Inc., catalogue no. 155C or 552C) or Nylon flocked swabs with liquid Amies (e.g. Copan Diagnostic Inc., catalogue no. 480CE)
- Heating Block at 95 °C
- Real-time PCR instrument:

Roche:	LightCycler® 480II
Agilent Technologies:	Mx3005P
Applied Biosystems:	ABI 7500
Abbott:	m2000rt
Bio-Rad:	CFX96™
Cepheid:	SmartCycler®
QIAGEN:	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit I (PG0001) for use with the LightCycler® 480
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves

7. Precautions for users

For *in-vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.
For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com

8. Sample collection and storage

8.1 Specimen collection

Moisten the swab with sterile saline or use dry swabs. Nasal specimen should be collected with the recommended swab (refer to section 6. Additional equipment and materials required) according to manufacturer's instruction.

8.2 Sample preparation

8.2.1 DNA Isolation from swabs

For DNA isolation from swabs the following procedure is recommended: Add 200 µl Lysis Buffer 1 into a preparation tube. Insert the swab in the pre-pipetted Lysis Buffer 1 and cut or break the swab stem. Cap the preparation tube tightly and vortex strongly for 60 seconds. Heat and shake the preparation tube at 95 °C for 10 minutes in a heating block. Centrifuge for 1 minute at 13,000 x g and apply the supernatant as sample.

Note: Repeat the centrifugation step in case of strong turbidity (if necessary).

The RIDA®GENE MRSA assay contains an Internal Control DNA (ICD), which can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as a PCR inhibition control.

If the ICD is used only as a PCR inhibition control, 1µl of the ICD should be added to the Master-Mix (see Tab.3).

If the ICD is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the ICD has to be added during extraction procedure. The ICD should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen.

8.2.2 DNA Isolation from Cultures

For DNA isolation from culture the following procedure is recommended: Add 200 µl Lysis Buffer 1 into a preparation tube. Collect colonies with an inoculation loop and suspend them in the pre-pipetted Lysis Buffer 1. Cut or break the inoculation loop stem. Cap the preparation tube tightly and vortex strongly for 60 seconds. Heat and shake the preparation tube at 95 °C for 10 minutes in a heating block. Centrifuge for 1 minute at 13,000 x g and apply the supernatant as sample.

Note: Repeat the centrifugation step in case of strong turbidity (if necessary).

The RIDA®GENE MRSA assay contains an Internal Control DNA (ICD), which can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as a PCR inhibition control.

If the ICD is used only as a PCR inhibition control, 1 µl of the ICD should be added to the Master-Mix (see Tab.3).

If the ICD is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the ICD has to be added during extraction procedure. The ICD should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and negative control must be included in each assay run.

We recommend to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab. 2, Tab. 3). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the PCR Water and the ICD before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 2: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 µl
	Total	**20.0 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit Code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl PCR Water as negative control to the pre-pipetted Master-Mix.

Note: *If the ICD is used as extraction control for the sample preparation procedure* ***and*** *as PCR inhibition control, we recommend to add 1 µl of the ICD to the negative control PCR-Mix.*

Sample: Add 5 µl DNA-Extract to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: *If the ICD is used as extraction control for the sample preparation procedure* ***and*** *as PCR inhibition control, we recommend to add 1 µl of the ICD to the positive control PCR-Mix.*

Note: *Use on the Rotor-Gene Q (QIAGEN) only 0,1 ml tubes.*

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument Set-up (see Tab. 4, Tab. 5).

9.3 PCR Instrument Set-up

Tab. 4: Real-time PCR profile for LightCyler® 480II, SmartCycler® and Rotor-Gene Q

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	10 sec, 95 °C
Annealing / Extension	15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: *Annealing and Extension occur in the same step.*

Note: ***Check that the "Manual Thres. Fluor Units" for Channel 1 is set to 10.0 and for Channel 2 to 4 is set to 5.0 on the SmartCycler® (Cepheid). Due to variations between different cyclers, it may be required to individually adapt the "Manual Thresh Fluor Units" for channel 1.***

Tab. 5: Real-time PCR profile for Mx3005P, ABI7500, m2000rt and CFX96™

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	15 sec, 95 °C
Annealing / Extension	30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: *Annealing and Extension occur in the same step.*

9.4 Detection Channel Set-up

Tab. 6: Selection of appropriate detection channels

Real-time PCR Instrument	Detection	Detection Channel	Note
Roche LightCycler® 480II	SCCmec / orfX junction	465/510	**RIDA®GENE Color Compensation Kit I (PG0001) is required**
	ICD	533/580	
	SA442 *(S. aureus)*	533/610	
	mecA / mecC	618/660	
Cepheid SmartCycler®	SCCmec / orfX junction	Channel 1	-
	ICD	Channel 2	
	SA442 *(S. aureus)*	Channel 3	
	mecA / mecC	Channel 4	
ABI 7500	SCCmec / orfX junction	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
	SA442 *(S. aureus)*	ROX	
	mecA / mecC	Cy5	
Abbott m2000rt	SCCmec / orfX junction	FAM	**-**
	ICD	VIC	
	SA442 *(S. aureus)*	ROX	
	mecA / mecC	Cy5	
Agilent Techn. Mx3005P	SCCmec / orfX junction	FAM	**Check that reference dye is none**
	ICD	HEX	
	SA442 *(S. aureus)*	ROX	
	mecA / mecC	Cy5	
Qiagen Rotor-Gene Q	SCCmec / orfX junction	Green	**The gain settings have to be set to 5**
	ICD	Yellow	
	SA442 *(S. aureus)*	Orange	
	mecA / mecC	Red	
Bio-Rad CFX96™	SCCmec / orfX junction	FAM	-
	ICD	VIC	
	SA442 *(S. aureus)*	ROX	
	mecA / mecC	Cy5	

*** Due to variations between different cyclers, it may be required to individually adapt the Manual Thres. Fluor Units" for channel 1.**

10. Quality Control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instructions. Positive and negative controls have to show correct results (see Table 7, Fig. 1, Fig. 2, Fig. 3) in order to determine a VALID run.
The positive control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Tab. 7: For a VALID run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target Ct
PTC	Positive	NA *[1]	See Quality Assurance Certificate
NTC	Negative	Ct > 20	0

*[1] *No Ct value is required for the ICD to make a positive call for the positive control.*

If the Positive Control (PTC) is not positive within the specified Ct range but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and a new Positive Control.

If the Negative Control (NTC) is not negative but the Positive control is valid prepare all new reactions using remaining purified nucleic acids and a new Negative Control.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instructions. Positive and negative controls have to show correct results (see Fig. 1).

Fig. 1: Correct run of the positive and negative control (SCCmec/orfX junction) on the LightCycler® 480II

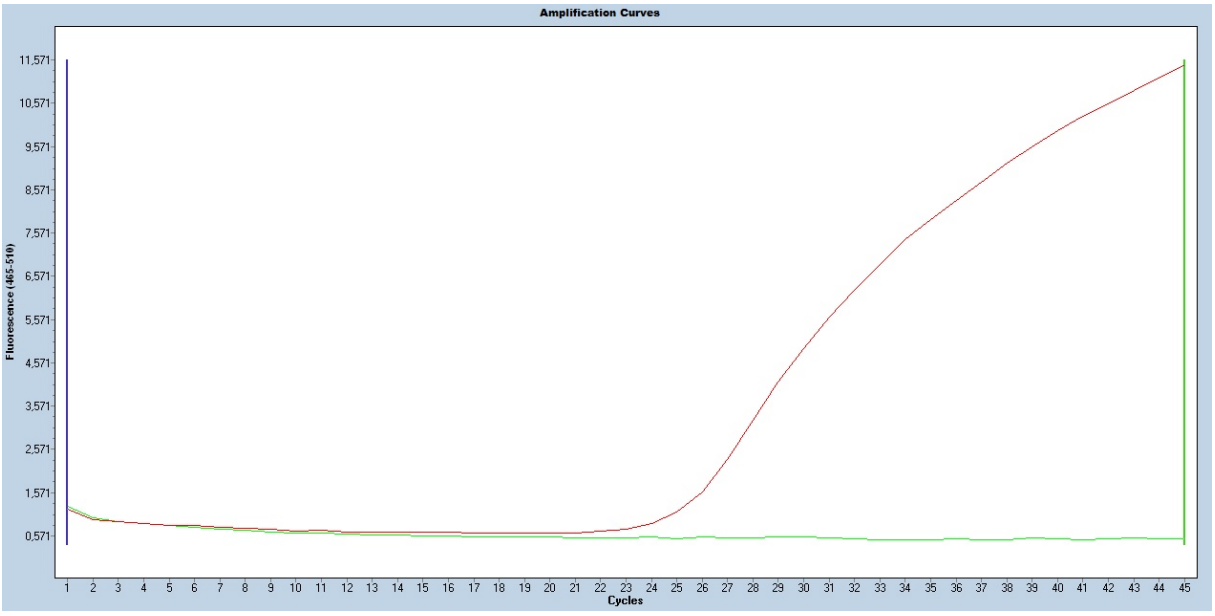


Fig. 2: Correct run of the positive and negative control (SA442 gene (*S. aureus*)) on the LightCycler® 480II

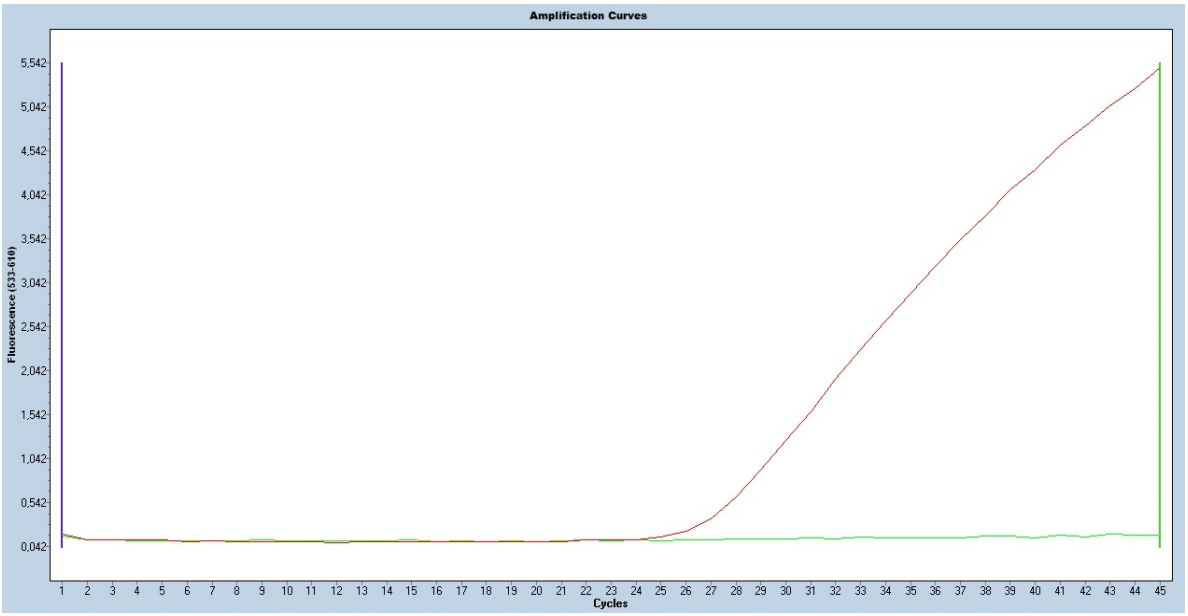
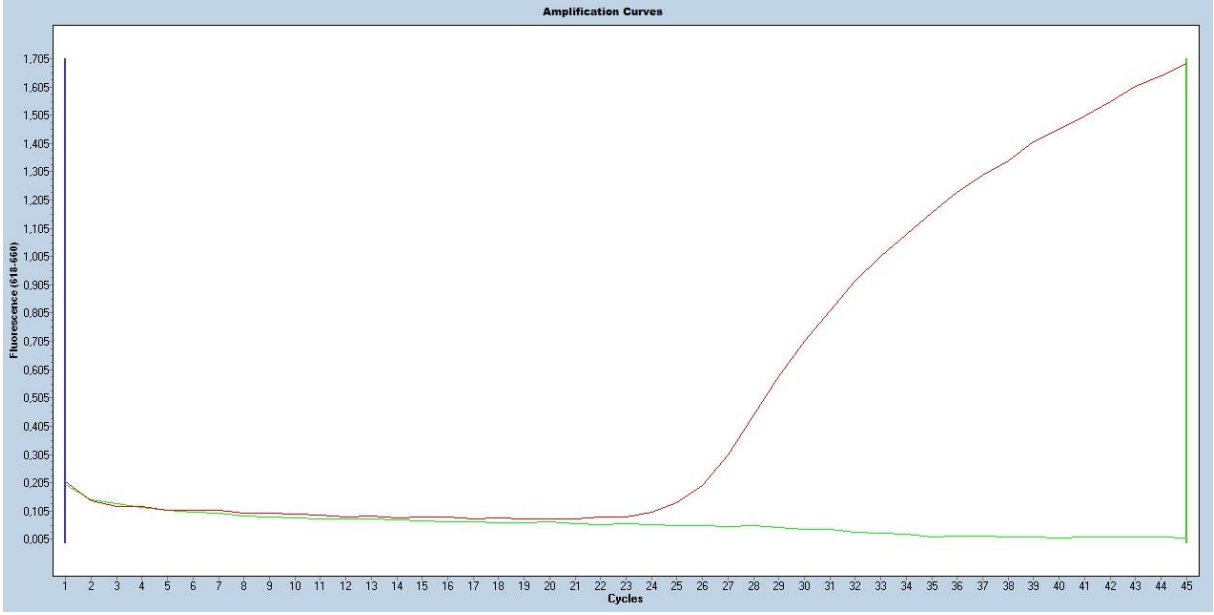


Fig. 3: Correct run of the positive and negative control (mecA/mecC gene) on the LightCycler® 480II



11. Evaluation and interpretation

The result interpretation is done according to Table 8.

Tab. 8: Sample interpretation

Target genes				
SCCmec / orfX junction	**mecA / mecC**	**SA442 *(S. aureus)***	**ICD**	**Result**
positive	**positive**	**positive**	**positive/negative**	**MRSA***
negative	**positive**	**positive**	**positive/negative**	**Mixed infection: MSSA** and CoNS***** (*m*ethicillin/oxacillin resistance)
positive	negative	**positive**	**positive/negative**	**MSSA****
negative	negative	**positive**	**positive/negative**	**MSSA****
negative	**positive**	negative	**positive/negative**	**CoNS***** (methicillin/oxacillin resistance)
negative	negative	negative	**positive**	**Negative** (Target genes are not detectable)
negative	negative	negative	negative	**Not evaluable**

* MRSA = Methicillin-resistant *S. aureus*

** MSSA = Methicillin-sensitive *S. aureus*

*** CoNS = Coagulase-negative *Staphylococcus*

A sample is evaluated negative, if the sample shows no amplification signal in the detection system, but the Internal Control DNA (ICD) is positive. An inhibition of the PCR reaction or a failure in the extraction procedure can be excluded by the detection of the Internal Control DNA (ICD).

A sample is evaluated positive, if both, the sample and the Internal Control DNA, (ICD) show an amplification signal in the detection system.

A sample is evaluated positive, if the sample shows an amplification signal in the detection system, but the Internal Control DNA (ICD) is negative. The detection of the internal amplification control is not necessary, because high concentrations of the amplicon can cause a weak or absent signal of the internal amplification control.

A sample is evaluated invalid, if both, the sample and the Internal Control DNA (ICD) show no amplification signal in the detection system. The sample contained a PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. For the application of PCR-based MRSA screening methods, country-specific regulations are valid for which the user is responsible for the application and implementation. For example, according to KRINKO in Germany, PCR-based MRSA screening methods should be taken as preliminary until the definite culture result. PCR-based screening methods, however, can be used as preliminary basis to derive hygienic measures within the hospital.
3. This test has been validated for use only with the here described human swab specimens and cultures.
4. Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MRSA variants resulting in a false negative result with the RIDA®GENE MRSA assay.
5. Eleven (11) SCCmec types have been described in the literature. RIDA®GENE MRSA is designed to detect the SCCmec types I, II, III, IV, V, VI, VII, IX, X and XI. RIDA®GENE MRSA may not detect other SCCmec types resulting in false negative results.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of MRSA DNA since the RIDA®GENE MRSA assay simultaneously detects the SCCmec/orfX junction, a specific *S. aureus* sequence located within the SA442 gene and the mecA (encodes the penicillin binding protein (PBP2a)) and mecC gene.
8. The RIDA®GENE MRSA assay does not give a positive result for borderline oxacillin-resistant *Staphylococcus aureus* (BORSA).
9. A RIDA®GENE MRSA positive result does not necessarily indicate treatment eradication failure since DNA may persists. A negative result following a previously positive test result may indicate treatment eradication success or may occur due to intermittent shedding.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE MRSA multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction for the SCCmec/orfX junction, the mecA/mecC gene and SA442 gene (*S. aureus*) respectively (s.Fig. 4, Fig. 5, Fig. 6).

Fig. 4: Dilution series SCCmec/orfX junction (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

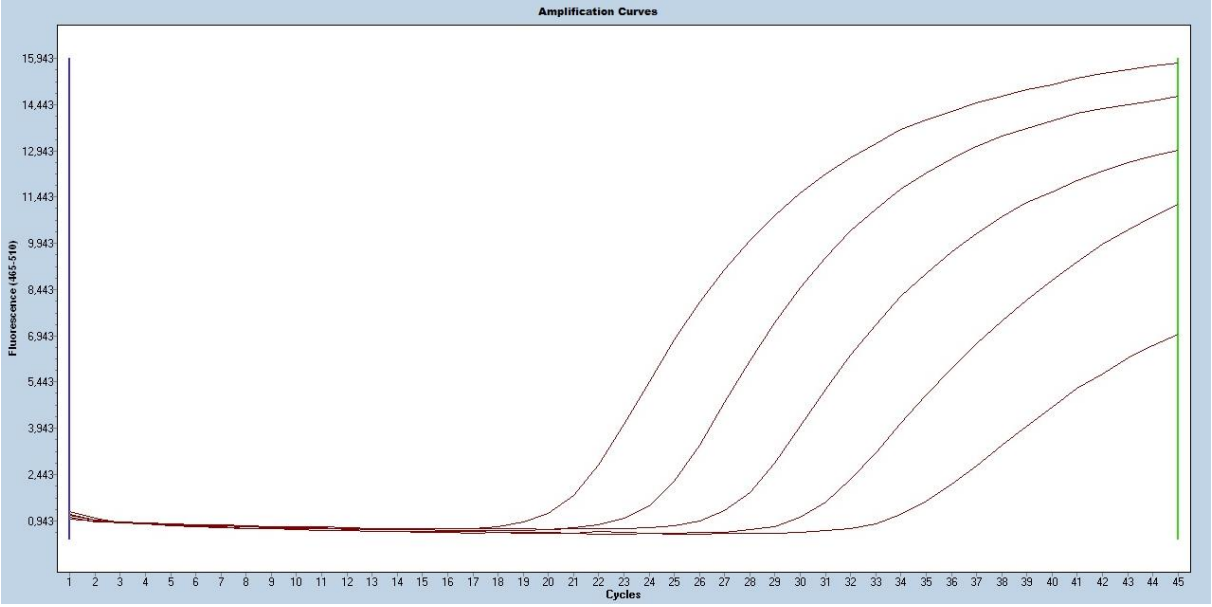


Fig. 5: Dilution series mecA/mecC gene (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

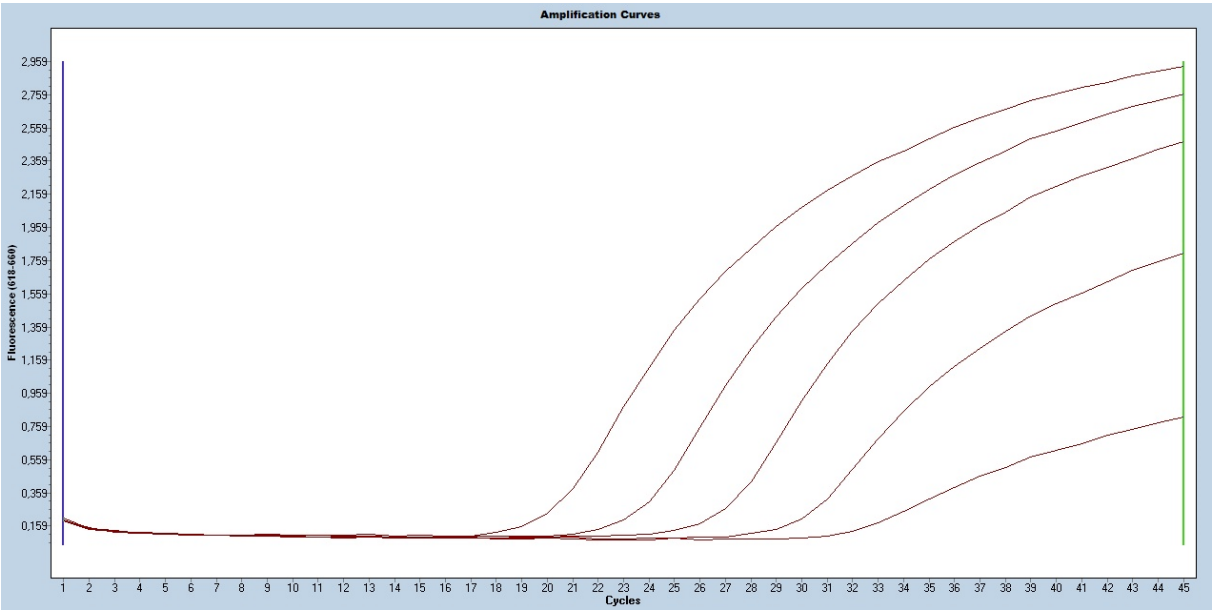
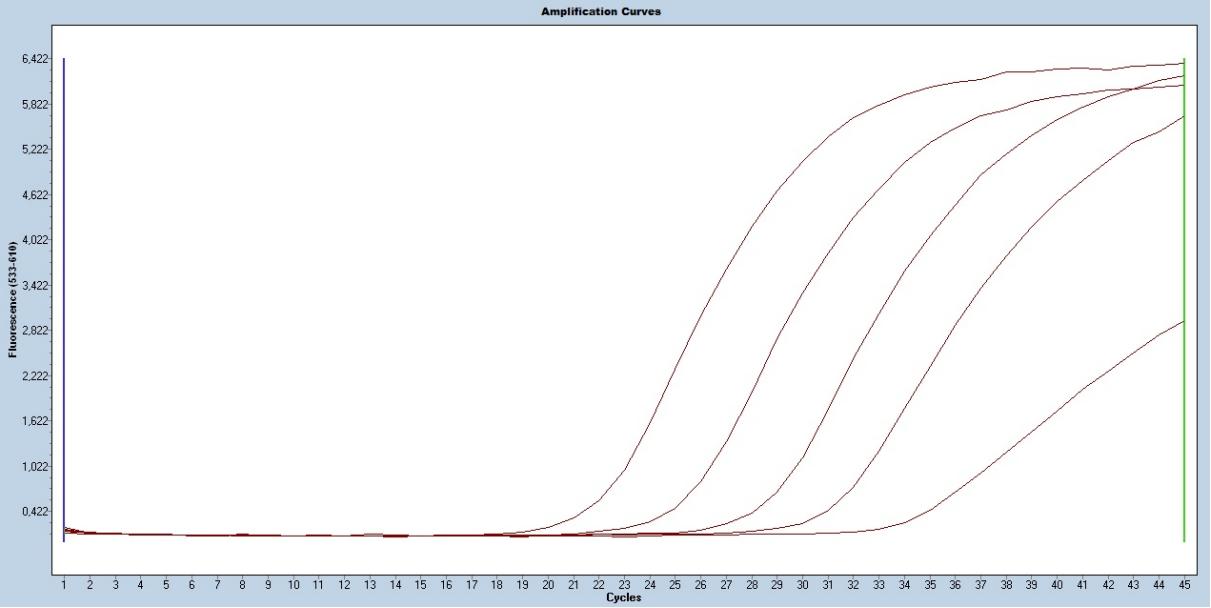


Fig. 6: Dilution series SA442 gene (*S. aureus*) (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II



The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

13.2 Analytical specificity

The analytical specificity of the RIDA®GENE MRSA assay was evaluated by testing a panel of non-staphylococcal species, methicillin-sensitive coagulase-negative *Staphylococci* (MSCoNS), methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS), borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA). All tested species were found negative for MRSA with the RIDA®GENE MRSA assay.

Tab. 8: Cross-reactivity testing

Non-staphylococcal species (number tested)					
Arcobacter butzleri (1)	-	*Clostridium sordellii* (1)	-	*Serratia liquefaciens* (1)	-
Aeromonas hydrophila (1)	-	Enteropathogenic *E.coli* (1)	-	*Shigella flexneri* (1)	-
Bacillus cereus (1)	-	Enterotoxic *E. coli* (1)	-	*Streptococcus agalactiae (Group B)* (1)	-
Bacteroides fragilis (1)	-	Shiga toxin-producing *E.coli* (1)	-	*Streptococcus equisimilis (Group C)* (1)	-
Campylobacter coli (1)	-	*Enterobacter cloacae* (1)	-	*Streptococcus mutans* (1)	-
Campylobacter jejuni (1)	-	*Enterococcus faecalis* (1)	-	*Streptococcus suis* (1)	-
Candida albicans (1)	-	*Klebsiella oxytoca (1)*	-	*Streptococcus pneumoniae* (1)	-
Citrobacter freundii (1)	-	*Pseudomonas aeruginosa* (1)	-	*Streptococcus pyogenes (Group A)* (1)	-
Clostridium difficile (1)		*Salmonella enteritidis* (1)		*Vibrio parahaemolyticus* (1)	-
Clostridium perfringens (1)		*Salmonella typhimurium* (1)		*Yersinia enterocolitica* (1)	-
Methicillin-sensitive coagluase-negative *Staphylococci* (number tested)					
S. epidermidis (5)	-	*S. simulans* (1)	-	*S. xylosus* (1)	-
S. hominis (5)	-	*S. pettenkoferi* (1)	-		
S. lugdunensis (2)	-	*S. warneri* (5)	-		
Methicillin-sensitive coagluase-positive *Staphylococci* (number tested)					
S. delphini (1)	-	*S. hyicus* (1)	-	*S. pseudointermedius* (1)	-
Methicillin-resistant coagluase-negative *Staphylococci* (number tested)					
S. haemolyticus (2)	-	*S. epidermidis* (13)	-	*S. capitis* (3)	-
Borderline oxacillin-resistant *Staphylococcus aureus* (1)					-
Methicillin-sensitive *Staphylococcus aureus* (9)					-

Explanation of Symbols

IVD	For *in vitro* diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
	Number of test
	Date of manufacture
	Manufacturer

Literature

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