RIDA®GENE MRSA LC2.0

real-time PCR

Art. No.: PG0625

100 reactions

For *in vitro* diagnostic use.

-20 °C

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE MRSA LC2.0 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 swabs and cultures on the LightCycler® 2.0. RIDA®GENE MRSA LC2.0 real-time multiplex PCR is intended for use as an aid in diagnosis of MRSA infection.

2. 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-aquired 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 healtcare 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 LC2.0 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 and SCCmec / orfX junction (types I, II, III, IV, V, VI, VII, IX, X, XI) specific for MRSA occurs (if present).
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 LC2.0 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	2 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: LightCycler® 2.0 (Roche)
- RIDA®GENE Color Compensation Kit II (PG0002)
- Real-time PCR consumables (reaction vials)
- 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 only.
- DNA isolation, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- 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.
- Calcium alginate swabs and swabs with wooden or aluminum shafts and / or cotton tips may inhibit PCR and can lead to false negative results. Use the recommended swabs for specimen collection.

8. Test procedure

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 nasal swab

For DNA isolation from nasal 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 12,000 rpm and apply the supernatant as sample.

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

The RIDA®GENE MRSA LC2.0 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 12,000 rpm and apply the supernatant as sample.

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

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8.3 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.

8.4 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial.

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.*

Cover reaction vials. Spin down and place in the LightCycler® 2.0. The PCR reaction should be started according to the PCR instrument Set-up (see Tab.4).

8.5 PCR Instrument Set-up

Tab.4: Real-time PCR profile

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.*

8.6 Detection Channel Set-up

Tab.5: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection Channel	Dark-Quencher	Note
Roche LightCycler® 2.0	mecA / mecC	705	+	**RIDA®GENE Color Compensation Kit II (PG0002) is required**
	SCCmec / orfX junction	530	+	
	ICD	560	+	

9. Result interpretation

The analysis of the samples is done by the software of the LightCycler® 2.0 according to the manufacturer`s instructions. Positive and negative controls have to show correct results (see Fig.1, Fig.2).

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.

Fig.1: Correct run of the positive and negative control (SCCmec / orfX junction) on the LightCycler® 2.0

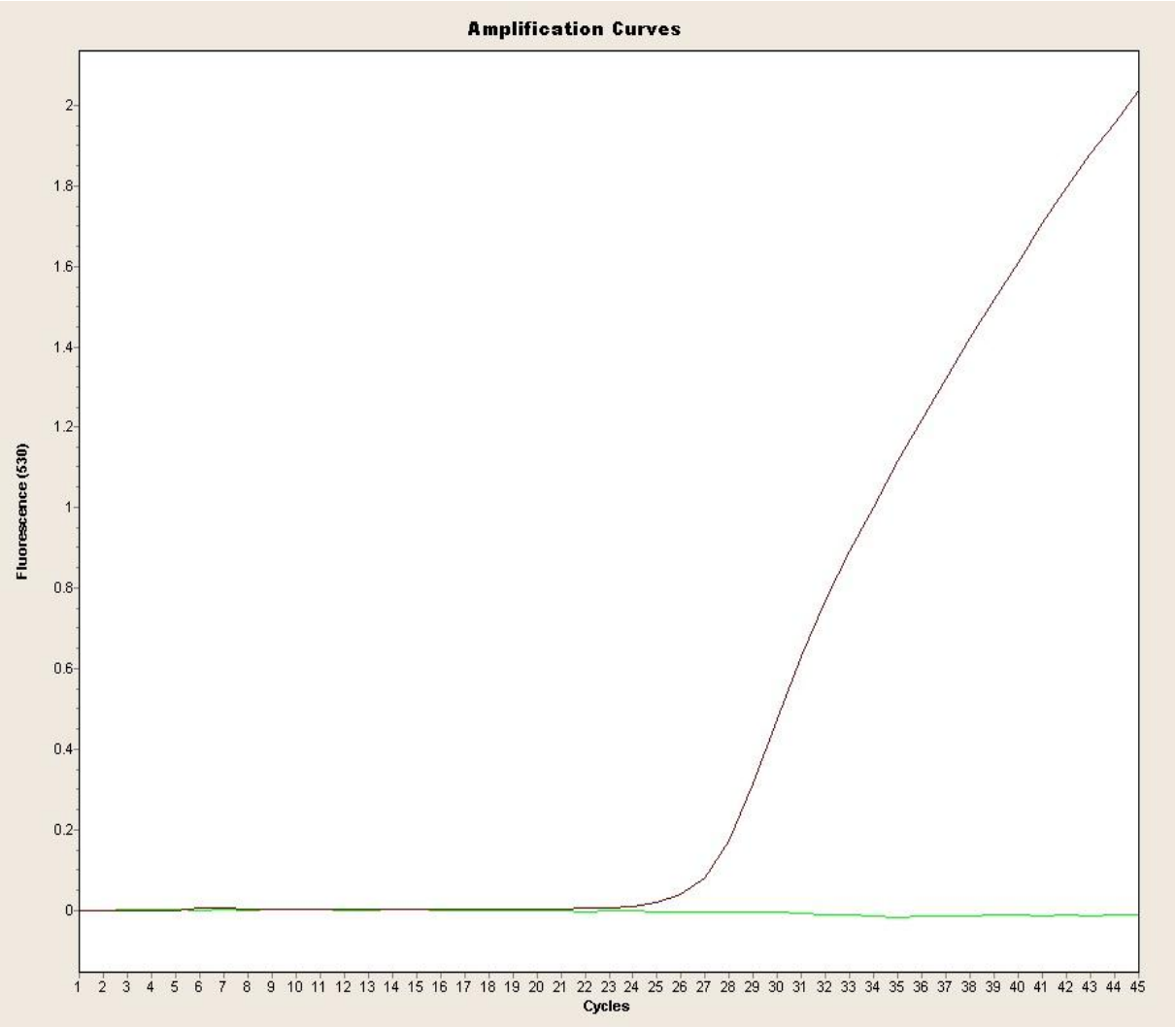
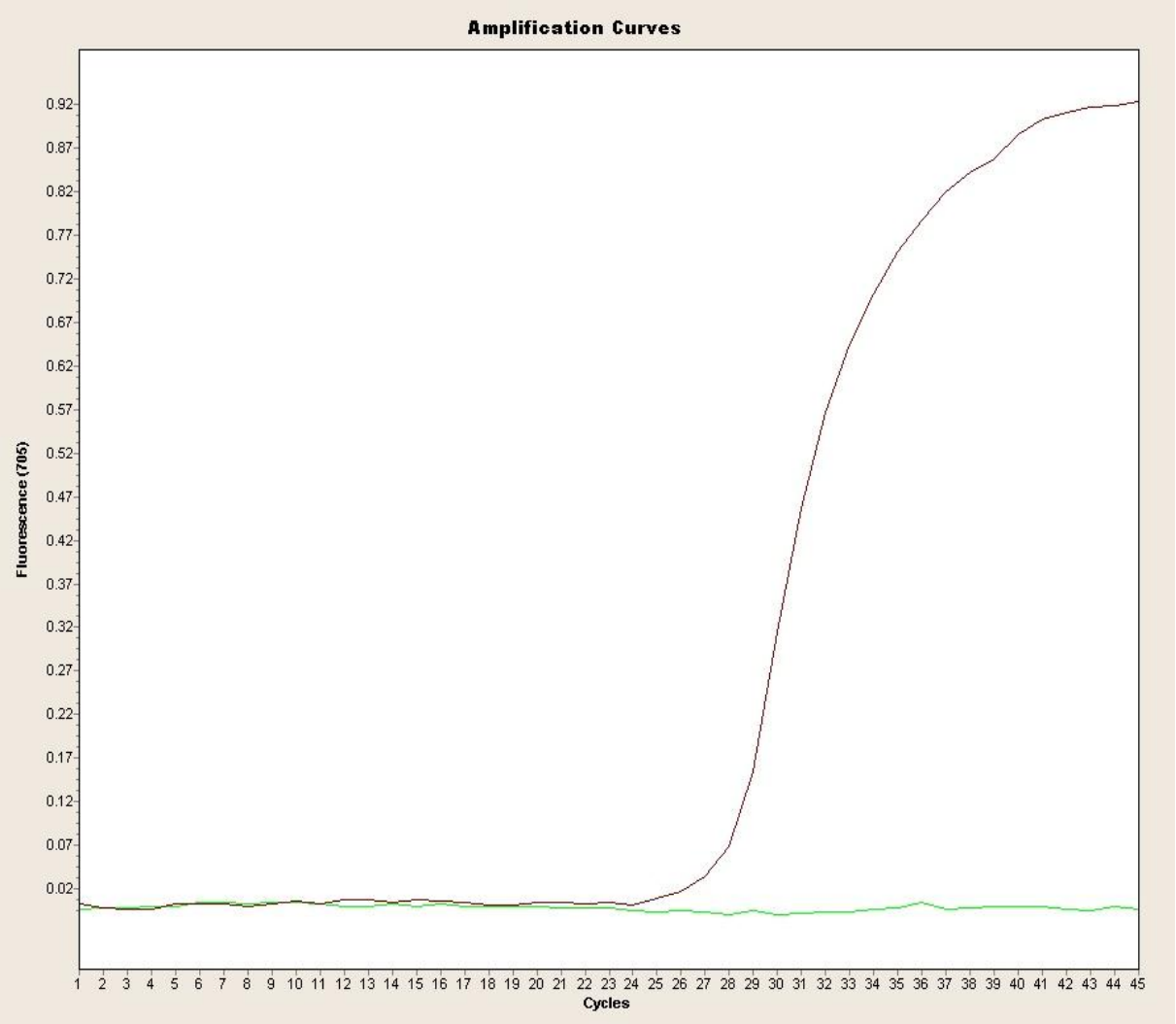


Fig.2: Correct run of the positive and negative control (mecA / mecC gene) on the LightCycler® 2.0



The result interpretation is done according to Table 6.

Tab.6: Sample interpretation

Target genes			
SCCmec / orfX junction	**mecA / mecC**	**ICD**	**Result**
positive	**positive**	**positive/negative**	**MRSA***
positive	negative	**positive/negative**	**MSSA****
negative	**positive**	**positive/negative**	**CoNS***** (methicillin-/oxacillin-resistance)
negative	negative	**positive**	**Negative** (Target genes are not detectable)
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.

10. Test characteristics

10.1 Analytical sensitivity

The RIDA®GENE MRSA LC2.0 multiplex real-time PCR has a detection limit of ≤ 5 DNA copies per reaction for the SCCmec / orfX junction and the mecA / mecC gene respectively (s.Fig.3, Fig.4).

Fig.3: Dilution series SCCmec / orfX junction (10^5 – 10^1 DNA copies per µl) on the LightCycler® 2.0

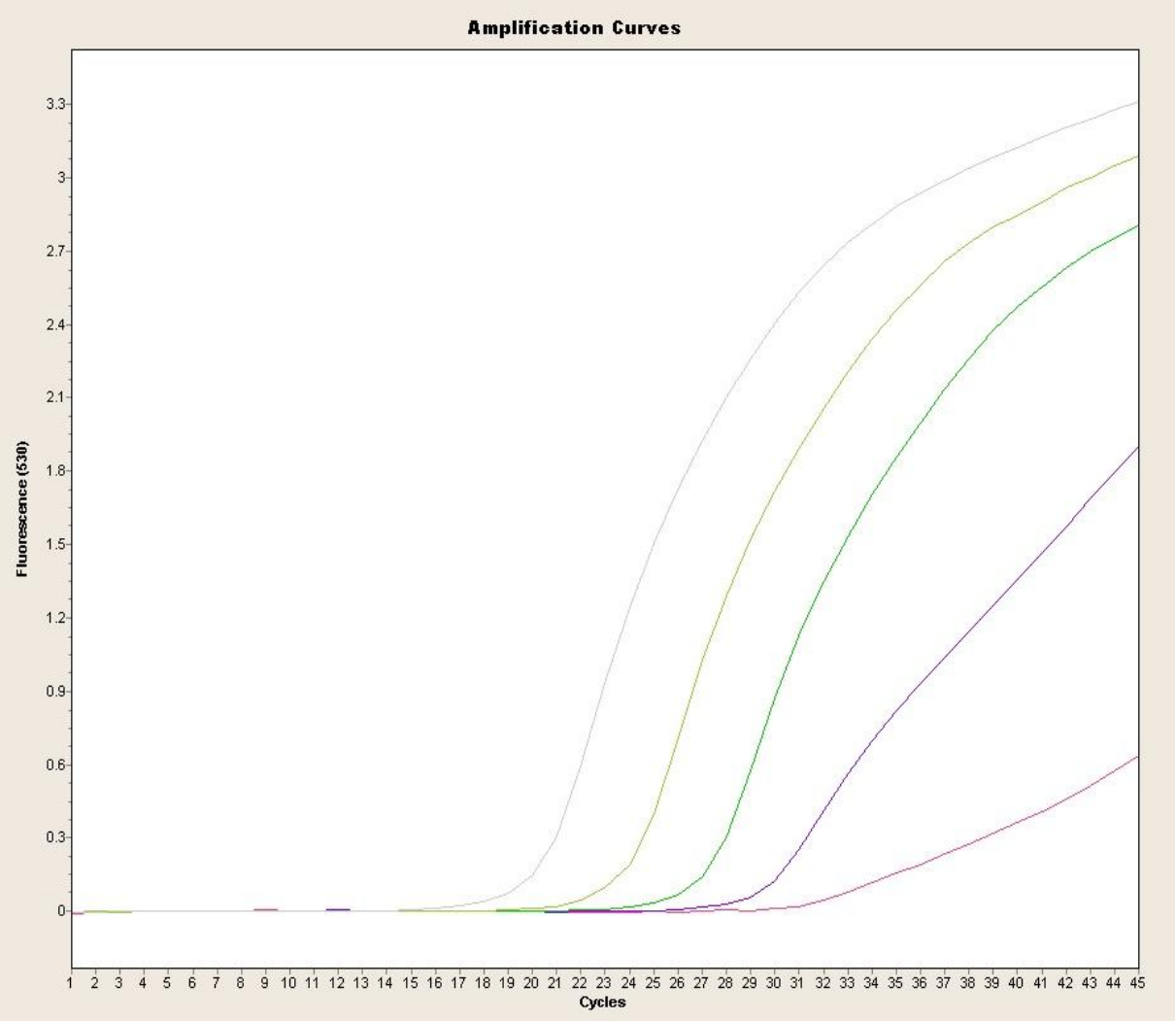
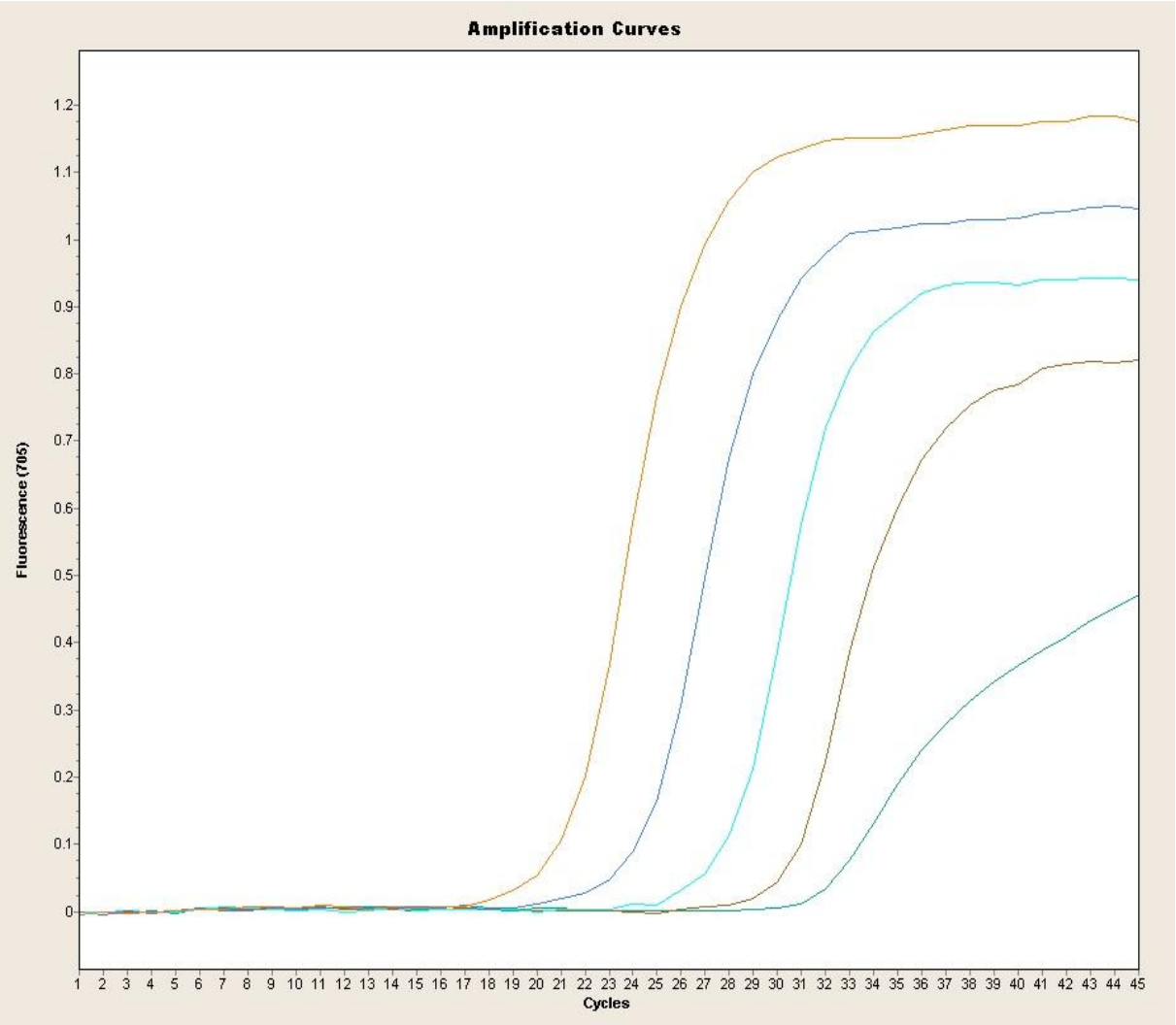


Fig.4: Dilution series mecA / mecC gene (10^5 – 10^1 DNA copies per µl) on the LightCycler® 2.0



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

10.2 Analytical specificity

The analytical specificity of the RIDA®GENE MRSA LC2.0 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) (s.Tab.7). All tested species were found negative for MRSA with the RIDA®GENE MRSA LC2.0 assay.

Tab.7: Cross-reactivity testing

Non-staphylococcal species (number tested)					
Arcobacter butzleri (1)	-	*Clostridium difficile* (1)	-	*Klebsiella oxytoca (1)*	-
Aeromonas hydrophila (1)	-	*Clostridium perfringens* (1)	-	*Pseudomonas aeruginosa* (1)	-
Bacillus cereus (1)	-	*Clostridium sordellii* (1)	-	*Salmonella enteritidis* (1)	-
Bacteroides fragilis (1)	-	Enteropathogenic *E.coli* (1)	-	*Salmonella typhimurium* (1)	-
Campylobacter coli (1)	-	Enterotoxic *E. coli* (1)	-	*Serratia liquefaciens* (1)	-
Campylobacter jejuni (1)	-	Shiga toxin-producing *E.coli* (1)	-	*Shigella flexneri* (1)	-
Candida albicans (1)	-	*Enterobacter cloacae* (1)	-	*Vibrio parahaemolyticus* (1)	-
Citrobacter freundii (1)	-	*Enterococcus faecalis* (1)	-	*Yersinia enterocolitica* (1)	-
Methicillin-sensitive coagluase-negative *Staphylococci* (number tested)					
S. epidermidis (4)	-	*S. warneri* (4)	-	*S. lugudensis* (2)	-
S. hominis (4)	-	*S. pettenkoferi* (1)	-		
Methicillin-resistant coagluase-negative *Staphylococci* (number tested)					
S. haemolyticus (2)	-	*S. epidermidis* (13)	-	*S. capitis* (2)	-
Borderline oxacillin-resistant *Staphylococcus aureus* (1)					-
Methicillin-sensitive *Staphylococcus aureus* (9)					-

11. 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. This test has been validated for use with only human nasal specimens and cultures.
3. 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 LC2.0 assay.
4. Eleven (11) SCCmec types have been described in the literature. The RIDA®GENE MRSA LC2.0 multiplex real-time PCR assay is designed to detect the SCCmec types I, II, III, IV, V, VI, VII, IX, X and XI. The RIDA®GENE MRSA LC2.0 assay may not detect other SCCmec types resulting in false negative results.
5. 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.
6. 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 LC2.0 assay simultaneously detects the SCCmec/orfX junction and the mecA (encodes the penicillin binding protein (PBP2a) and mecC gene.
7. A RIDA®GENE MRSA LC2.0 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.

12. Literature

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