Instruction for Use

diarellaMRSA seqc

real time PCR Kit

For *in vitro* detection of the DNA of Methicillin Resistant Staphylococcus aureus (MRSA) extracted from biological specimens.

REF **G01140-96**

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- 18°C

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1 Intended Use

The diarellaMRSA seqc real time PCR is an assay for the detection of the DNA of *MRSA* extracted from biological specimens.

2 Pathogen Information

Staphylococcus aureus are gram-positive coccal bacteria which are ubiquitously found in the environment. About 25-30 % of the human population are long-term carriers of *S. aureus* because the bacteria are frequently part of the skin flora found in the nose and on skin. *S. aureus* can cause a range of illnesses such as minor skin infections, like furuncles and abscesses, pyomyositis, but also life-threatening diseases such as pneumonia, endocarditis, toxic shock syndrome (TSS), and sepsis.

Of increasing importance worldwide are Methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Especially in hospitals MRSA present a danger, because they are resistant to all ß-lactam antibiotics (e.g. penicillin) and often possess further resistances to other antibiotics.

3 Principle of the Test

The diarellaMRSA seqc real time PCR contains specific primers and dual-labeled probes for the amplification of the DNA of MRSA extracted from biological specimens.

The PCR targets the SCCmec/orfX junction and allows the detection of MRSA in biological samples, even those containing Coagulase-Negative Staphylococci. Furthermore, diarellaMRSA seqc real time PCR Kit allows the detection of the methicillin resistance gene mecA/mecC, to eliminate false positive results through dropout mutants.

The presence of nucleic acid is detected by an increase in fluorescence due to hydrolysis of the probes during amplification. The fluorescence of the pathogen-specific probes is measured in the FAM channel. The fluorescence of the mecA/mecC gene specific probes is measured in the Cy5 channel. For a positive MRSA result, both channels need to show an amplification.

Furthermore, diarellaMRSA seqc real time PCR contains a Control DNA (Internal Process Control, IPC), which is added during DNA extraction and detected in the same reaction by a HEX-labeled probe.

The Control DNA allows the detection of PCR inhibition and acts as control, that the nucleic acid was isolated from the biological specimen.

Additionally, diarellaMRSA seqc real time PCR Kit contains an Internal System Control (ISC). The ISC consists of primers and probes for the detection of a house keeping gene (Succinate dehydrogenase) in the eluate from human biological specimens. The ISC helps preventing false negative results due to insufficient sample drawing or transport. The amplification of the Succinate dehydrogenase target sequence is measured in the ROX channel.

4 Package Contents

The reagents supplied are sufficient for 96 reactions.

Table 1: Components of the diarellaMRSA seqc real time PCR Kit

Label	Lid Colour	Content 96
Reaction Mix	yellow	1 x 1344 µl
Positive Control	red	1 x 150 µl
Negative Control	green	1 x 150 µl
Control DNA	colourless	1 x 480 µl

5 Equipment and Reagents to be Supplied by User

- DNA isolation kit (e.g. NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

6 Transport, Storage and Stability

The diarellaMRSA seqc real time PCR Kit is shipped on dry ice or cool packs. All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. Up to 20 freeze and thaw cycles are possible. For convenience, opened reagents can be stored at +2-8°C for up to 6 months.

Protect kit components from direct sunlight during the complete test run.

7 Warnings and Precautions

Read the Instructions for Use carefully before using the product.
Before first use check the product and its components for:

- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.
- Do not combine kit components of different lot numbers.

8 Sample Material

Starting material for diarellaMRSA seqc real time PCR is DNA isolated from biological specimens.

9 Sample Preparation

Commercial kits for DNA isolation such as the following are recommended:

- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

Please follow the instructions for use of the respective extraction kit.

Important:

In addition to the samples always run a ‚water control' in your extraction. Treat this water control analogous to a sample.

Comparing the amplification of the Control DNA in the samples to the amplification of the internal control in the water control will give insights on possible inhibitions of the real time PCR. Furthermore, possible contaminations during DNA extraction will be detectable.

Please note the chapter ‚Control DNA'.

If the real time PCR is not performed immediately, store extracted DNA according to the instructions given by the manufacturer.

10 Control DNA

A Control DNA is supplied as extraction control. This allows the user to control the DNA isolation procedure and to check for possible real time PCR inhibition.

Add 5 µl Control DNA per extraction (5 µl x (N+1)). Mix well. Perform the DNA isolation according to the manufacturer's instructions.

The Control DNA must be added to the Lysis Buffer of the extraction kit.

11 Real time PCR

11.1 Important Points Before Starting:

- Please pay attention to the chapter 7 ‚Warnings and Precautions'.
- Before setting up the real time PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every PCR run one Positive Control and one Negative Control should be included.

- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed, and centrifuged very briefly.

11.2 Procedure

The Master Mix contains all of the components needed for PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the Master Mix

Volume per Reaction	Volume Master Mix
14.0 µl Reaction Mix	14.0 µl x (N+1)

Real time PCR set-up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument / take an optical PCR reaction plate.
- Pipet **14 µl** of the Master Mix into each optical PCR reaction tube / the optical PCR reaction plate.
- Add **6 µl** of the eluates from the DNA isolation (including the eluate of the water control), the Positive Control and the Negative Control to the corresponding optical PCR reaction tube / the optical PCR reaction plate (Table 3).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 3: Preparation of the real time PCR

Component	Volume
Master Mix	14.0 µl
Sample	6.0 µl
Total Volume	20.0 µl

11.3 Instrument Settings

For the real time PCR use one of the thermal profiles shown in Table 4 and Table 5.

Table 4: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	45
Annealing and Extension	40 sec	60°C	
	Acquisition at the end of this step		

If in the same run samples should be tested for pathogens with RNA genome, use the thermal profile shown in Table 5.

Table 5: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Reverse Transcription	10 min	45°C	1
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	45
Annealing and Extension	40 sec	60°C	
	Acquisition at the end of this step		

Dependent on the real time instrument used, further instrument settings have to be adjusted according to Table 6.

Table 6: Overview of the instrument settings required for the diarellaMRSA seqc real time PCR.

Real time PCR Instrument	Parameter	Detection Channel	Notes		
LightCycler 480II			Colour Compensation Kit Multiplex 1 (G070MP1-CC) required		
			Melt Factor	Quant Factor	Max Integration Time (sec)
	SCCmec/orfX	465-510	1	10	1
	Control DNA (IPC)	533-580	1	10	2
	ISC	533-610	1	10	2
	mecA/mecC Mutation/Deletion	618-660	1	10	3
Stratagene Mx3000P / Mx3005P	SCCmec/orfX	FAM	Gain 8	Reference Dye: None	
	Control DNA (IPC)	HEX	Gain 1		
	ISC	ROX	Gain 1		
	mecA/mecC Mutation/Deletion	Cy5	Gain 4		
QuantStudio 5 Bio-Rad CFX96 Bio-Rad CFX Opus 96	SCCmec/orfX	FAM	Option Reference Dye ROX: NO		
	Control DNA (IPC)	HEX			
	ISC	ROX			
	mecA/mecC Mutation/Deletion	Cy5			
Mic qPCR Cycler	SCCmec/orfX	Green	Gain 8		
	Control DNA (IPC)	Yellow	Gain 10		
	ISC	Orange	Gain 10		
	mecA/mecC Mutation/Deletion	Red	Gain 10		

12 Data Analysis

Following results can occur:

Signal/C_T Values				
FAM Channel SCCmec/orfX	**Cy5 Channel** resistance gene mecA/mecC	**ROX Channel** ISC	**HEX Channel** IPC	**Interpretation**
positive	**positive**	positive or negative[1]	positive or negative[2]	**Positive result, the sample contains *MRSA* DNA.**
positive	negative	positive or negative[1]	positive or negative[2]	**Negative result, the sample contains MS-*MRSA* DNA.**
negative	**positive**	positive or negative[1]	positive or negative[2]	**Negative result, the sample contains MR-CoNS DNA.**
negative	negative	positive	≤ 34 [3]	**Negative result, the sample contains no *MRSA/ MS-MRSA and MR-CoNS* DNA.**
negative	negative	negative	negative or > 34 [3]	**No diagnostic statement can be made.** The real time PCR is either inhibited or errors occurred while DNA extraction.

[1] If the analysed samples originate from cultivation, the ROX channel may be negative.

[2] A strong positive signal in the FAM or the Cy5 can inhibit the IPC. In such cases the result for the Control DNA can be neglected.

[3] In case of high C_T values, the IPC should be compared to the water extraction control as described in the chapter 'Assay Validation'.

Figure 1 to 4 show examples for positive and negative real time PCR results.

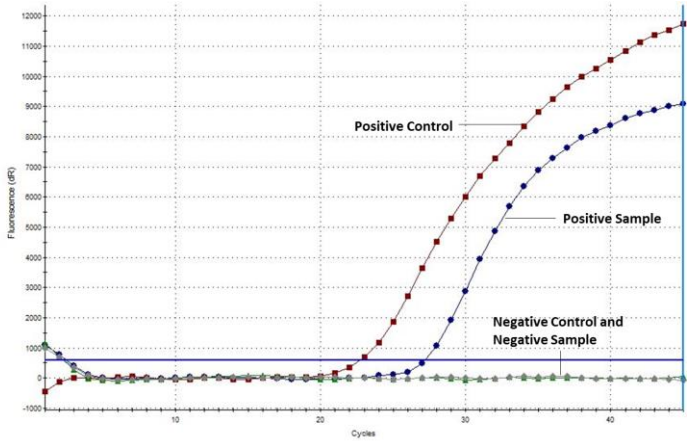


Figure 1: The Positive Sample shows pathogen specific amplification in the FAM channel (Positive Sample and Positive Control), whereas no fluorescence signal is detected in the Negative Sample or the Negative Control (Mx3005P).

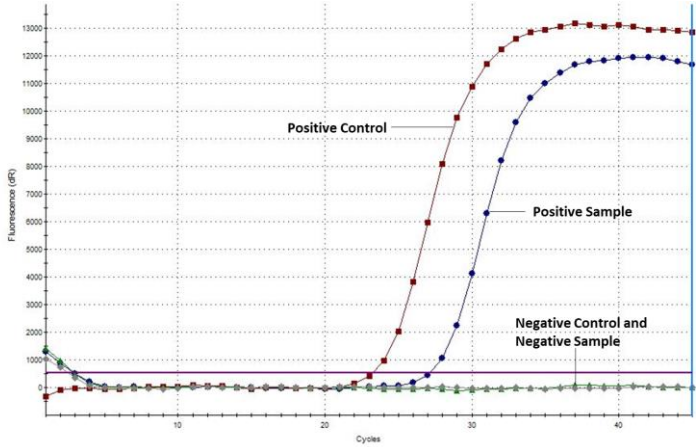


Figure 2: The Positive Sample shows pathogen specific amplification in the Cy5 channel (Positive Sample and Positive Control), whereas no fluorescence signal is detected in the Negative Sample or the Negative Control (Mx3005P).

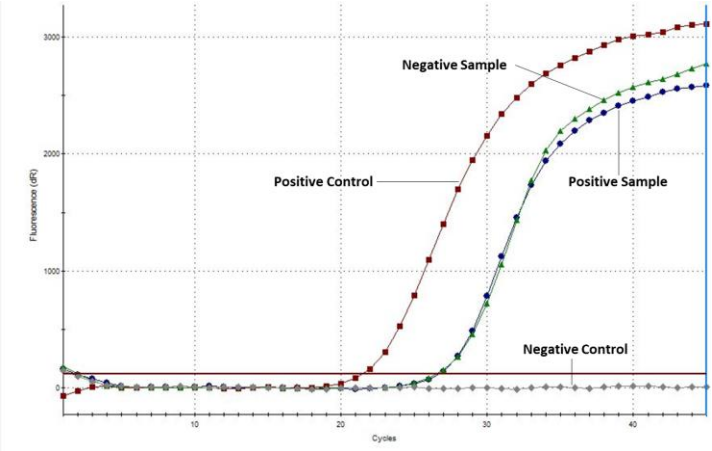


Figure 3: Signals of the amplification of the ISC in the ROX channel. The amplification signal in the positive and the negative sample indicates a sufficient amount of DNA in the sample eluate and confirms the integrity of the sampling. (Mx3005P)

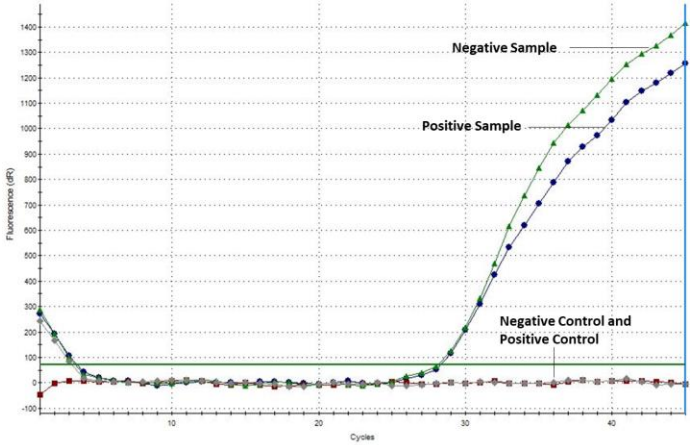


Figure 4: The Positive Sample and the Negative Sample show a signal in the Control DNA specific HEX channel (IPC). The amplification signal of the Control DNA in the Negative Sample shows that the missing signals in the pathogen specific channels FAM and Cy5 are not due to PCR inhibition or failure of DNA isolation, but that the sample is a true Negative Sample (Mx3005P).

13 Assay Validation

Negative Controls
The Negative Control must show no C_T in the FAM, Cy5, ROX and HEX channel.

Positive Controls
The Positive Control must show a positive (i.e. exponential) amplification curve in the different channels FAM, Cy5 and ROX. The Positive Control must fall below C_T 30.

Internal Controls
The following values for the amplification of the internal controls are valid using gerbion nucleic acid extraction kit NukEx Mag RNA/DNA. All internal controls (ISC and IPC, seqc – sample and extraction quality control) must show a positive (i.e. exponential) amplification curve.
The Control DNA (IPC) must fall below a C_T of 34. If the Control DNA is above C_T 34 this points to a purification problem or a strong positive sample that can inhibit the IPC. In the latter case, the assay is valid. It is recommended to perform the extraction of a water control in each run. The IPC in the water control must fall below a C_T of 34.
For accurately drawn respiratory swab samples, the ISC shows C_T values from app. 15 to app. 28. A heavily delayed signal of higher than a C_T of 34 indicates a low sample amount. Therefore, false negative results cannot be ruled out. In case of no amplifications neither in the FAM nor in the Cy5 channel, there must be an amplification curve in the ROX channel (ISC) and the HEX (IPC) channel when using eluates of primary samples from humans.
If other nucleic acid extraction kits are used, the customer must define own cutoffs. In this case the C_T value of the Control DNA (IPC) in an eluate from a sample should not be delayed for more than 4 C_T in comparison to an eluate from an extracted water control.

14 Limitations of the Method

- Strict compliance with the Instruction for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the diarellaMRSA seqc real time PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

15 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

No fluorescence signal in the FAM, Cy5, ROX channel of the Positive Control	
The selected channel for analysis does not comply with the protocol	Select the FAM channel for analysis of the *MRSA* specific amplification, the Cy5 channel for the mecA/mecC specific amplification, the ROX channel for the amplification of the ISC and the HEX channel for the amplification of the Control DNA (IPC).
Incorrect configuration of the real time PCR	Check your work steps and compare with chapter 'Procedure'.
The programming of the thermal profile is incorrect	Compare the thermal profile with the protocol in chapter 'Real time PCR'.
Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability'.
Weak or no signal of the Control DNA (IPC) and ISC and simultaneous absence of a signal in the bacteria specific FAM and/or Cy5 channel.	
real time PCR conditions do not comply with the protocol	Check the real time PCR conditions (chapter 'Real time PCR').
real time PCR inhibited	Make sure that you use an appropriate isolation method (see chapter 'Sample Preparation') and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed.
sample material not sufficient	Make sure that enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter 'Sample Preparation' and follow the manufacturer's instructions.
DNA loss during isolation process	In case the Control DNA was added before extraction, the lack of an amplification signal can indicate that the DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability'.
Detection of a fluorescence signal in the FAM and/or Cy5 channel of the Negative Control	
Contamination during preparation of the PCR	Repeat the real time PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after

	adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time PCR.
Detection of a fluorescence signal in the ROX channel of the Negative Control	
Contamination with human DNA during preparation of the real time PCR	As long as the ROX channel shows very high Ct values, the contamination is negligible. If the FAM and Cy5 channel are negative in the Negative Control, the PCR is still valid for the detection of MRSA.

16 Kit Performance

16.1 Analytical Sensitivity

For the FAM and Cy5 channel the limit of detection (LoD) of diarellaMRSA seqc real time PCR Kit was determined using serial dilutions of the AcroMetrix MRSA Positive Control, CE-IVD. Since there is no ISC within the AcroMetrix MRSA Positive Control, CE-IVD, the LoD for the ROX channel was determined using serial dilutions of a synthetic DNA-fragment containing the specific gene target sequence. The determination of the LoD was done on a Stratagene Mx3005P.

The LoD of diarellaMRSA seqc real time PCR Kit is $\leq$ 2.5 genome copies per µl for the FAM, Cy5 and ROX channel.

16.2 Analytical Specificity

The specificity of the diarellaMRSA seqc real time PCR was evaluated with different ring trial samples of known status and different other relevant viruses and bacteria found in biological samples and basing on in silico analyses.
All ring trial samples were detected correctly. Results are shown in Table 7 and Table 8.
The results for the sample analysis are shown Table 9 and Table 10. The results for the in silico analysis are shown in Table 11.

Table 7: Ring trial samples tested for the validation of the sensitivity of the diarellaMRSA seqc real time PCR Kit. Results for the FAM channel.

sample	expected result	result
cMRSA (S. aureus, oxaR, PVL-pos, spa:t 008)	**positive**	**positive**
MRSA (S. aureus, oxaR, PVL-neg)	**positive**	**positive**
Escherichia coli K12	negative	negative
cMSSA + CoNS (S. aureus, S. epidermidis oxaR, PVL-pos)	negative	negative
CoNS (S. epidermidis, oxaS)	negative	negative
MSSA + CoNS (S. aureus, S. hämolyticus oxaR, PVL-neg)	negative	negative
MRSA SCCmec TypV (S. aureus, oxaR, PVL-neg)	**positive**	**positive**
cMRSA (S. aureus, oxaR, PVL-pos)	**positive**	**positive**
MRSA (S. aureus, PVL-neg, pSA442 neg)	**positive**	**positive**
CoNS oxaS	negative	negative
cMRSA (S. aureus, oxaR, PVL-pos)	**positive**	**positive**
cMRSA (S. aureus, oxaR, PVL-pos)	**positive**	**positive**

Table 8: Ring trial samples tested for the validation of the sensitivity of the diarellaMRSA seqc real time PCR Kit. Results for the Cy5 channel.

sample	expected result	result
cMRSA (S. aureus, oxaR, PVL-pos, spa:t 008)	**positive**	**positive**
MRSA (S. aureus, oxaR, PVL-neg)	**positive**	**positive**
Escherichia coli K12	negative	negative
cMSSA + CoNS (S. aureus, S. epidermidis oxaR, PVL-pos)	**positive**	**positive**
CoNS (S. epidermidis, oxaS)	negative	negative
MSSA + CoNS (S. aureus, S. hämolyticus oxaR, PVL-neg)	**positive**	**positive**
MRSA SCCmec TypV (S. aureus, oxaR, PVL-neg)	**positive**	**positive**
cMRSA (S. aureus, oxaR, PVL-pos)	**positive**	**positive**
MRSA (S. aureus, PVL-neg, pSA442 neg)	**positive**	**positive**
CoNS oxaS	negative	negative
cMRSA (S. aureus, oxaR, PVL-pos)	**positive**	**positive**
cMRSA (S. aureus, oxaR, PVL-pos)	negative	negative

Table 9: Eluted DNA/RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of diarellaMRSA seqc real time PCR Kit, FAM channel.

sample	expected result FAM channel	diarellaMRSA seqc FAM channel
Pneumocystis jirovecii	negative	negative
Streptococcus agalactiae	negative	negative
Coxsackie A9 Strain P.B.	negative	negative
Herpes simplex Virus Type 2 Str. G	negative	negative
Borrelia burgdorferi	negative	negative
Staphylococcus ueberis	negative	negative
Streptococcus dysagalactiae	negative	negative
Entrococcus faecalis	negative	negative
Klebsiella	negative	negative
Staphylococcus intermedius	negative	negative
Pseudomonas aeruginosa	negative	negative
Staphylococcus sciuri	negative	negative
Legionela pneumophila	negative	negative
TBE Virus K617	negative	negative
Influenza A Virus (H1N1)	negative	negative
Influenza B Virus	negative	negative
Respiratory Syncytial Virus A	negative	negative
Respiratory Syncytial Virus B	negative	negative
Cytomegalie Virus AD169	negative	negative

Table 10: Eluted DNA/RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of diarellaMRSA seqc real time PCR Kit, Cy5 channel.

sample	**expected result Cy5 channel**	**diarellaMRSA seqc Cy5 channel**
Pneumocystis jirovecii	negative	negative
Streptococcus agalactiae	negative	negative
Coxsackie A9 Strain P.B.	negative	negative
Herpes simplex Virus Type 2 Str. G	negative	negative
Borrelia burgdorferi	negative	negative
Staphylococcus ueberis	negative	negative
Streptococcus dysagalactiae	negative	negative
Entrococcus faecalis	negative	negative
Klebsiella	negative	negative
Staphylococcus intermedius	negative	negative
Pseudomonas aeruginosa	negative	negative
Staphylococcus sciuri	negative	negative
Legionela pneumophila	negative	negative
TBE Virus K617	negative	negative
Influenza A Virus (H1N1)	negative	negative
Influenza B Virus	negative	negative
Respiratory Syncytial Virus A	negative	negative
Respiratory Syncytial Virus B	negative	negative
Cytomegalie Virus AD169	negative	negative

Table 11: Inclusivity of the diarellaMRSA seqc real time PCR Kit Primers and Probes (in silico analysis).

9 - 1000 whole genome sequences		Homology	Comment
SCCmec / orfX junction	forward Primer Mix	9 / 9 - 593 / 593	based on different SCCmec cassettes
	reverse Primer	1000 / 1000	only orfX
	Probe	1000 / 1000	only orfX
mec A	forward Primer	789 / 789	
	reverse Primer	789 / 789	
	Probe	789 / 789	
mec C	forward Primer	18 / 18	
	reverse Primer	18 / 18	
	Probe	18 / 18	
Succinate dehydrogenase	forward Primer	1000 / 1000	
	reverse Primer	1000 / 1000	
	Probe	1000 / 1000	

16.3 Linear Range

The linear range of the diarellaMRSA seqc real time PCR Kit was evaluated by analysing logarithmic dilution series of in vitro transcripts of the target sequences.

Figure 5: Determination of the linear range of the diarellaMRSA seqc real time PCR Kit for SCCmec in the FAM channel:

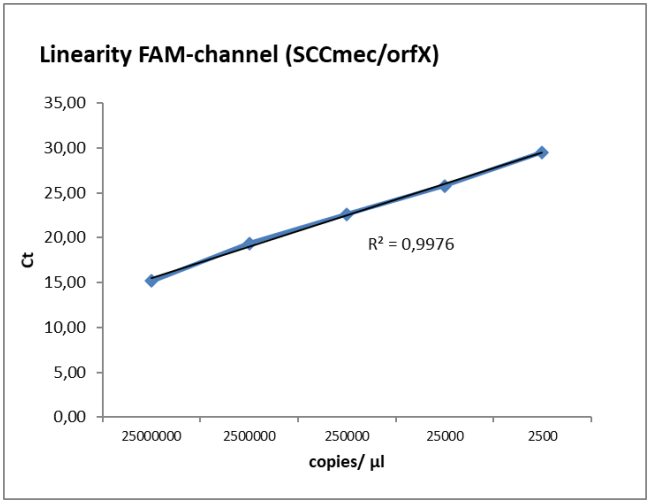


Figure 6: Determination of the linear range of the diarellaMRSA seqc real time PCR Kit for mecA/mecC in the Cy5 channel:

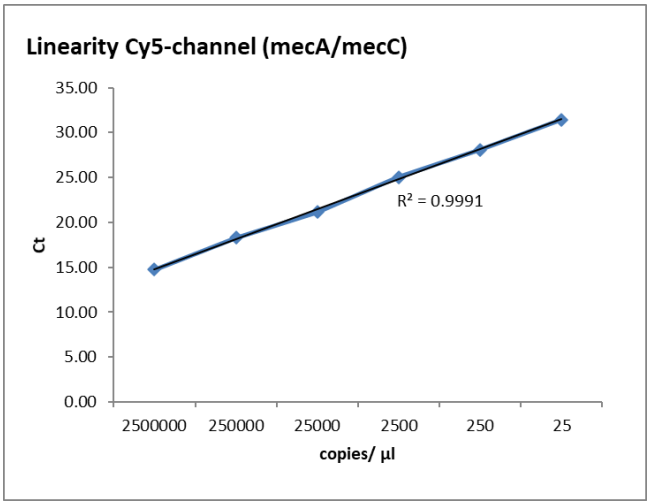
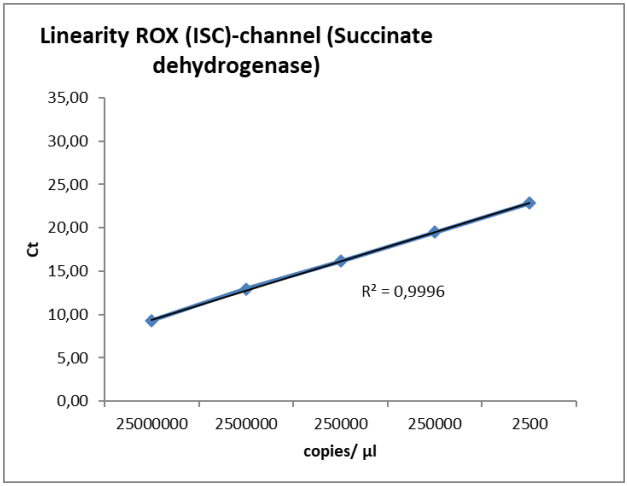


Figure 7: Determination of the linear range of the diarellaMRSA seqc real time PCR Kit for the ISC in the ROX channel:



16.4 Precision

The precision of the diarellaMRSA seqc real time PCR Kit was determined as intra-assay variability, inter-assay variability and inter-lot variability.

Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of SCCmec specific synthetic DNA, mecA/mecC specific synthetic DNA, ISC specific synthetic DNA and on the threshold cycle of the Control DNA (IPC). The results are shown in Table 12.

Table 12: Precision of the diarellaMRSA seqc real time PCR Kit

SCCmec (FAM)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	250	0.33	0.99
Inter-Assay-Variability	250	0.47	1.39
Inter-Lot-Variability	250	0.05	0.14
mecA/mecC (Cy5)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.25	0.78
Inter-Assay-Variability	25	0.23	0.72
Inter-Lot-Variability	25	0.17	0.52
ISC (ROX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	2.5	0.22	0.69
Inter-Assay-Variability	2.5	0.20	0.62
Inter-Lot-Variability	2.5	0.22	0.67
IPC (HEX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	250	0.34	1.20
Inter-Assay-Variability	250	0.85	2.96
Inter-Lot-Variability	250	0.26	0.91

16.5 Diagnostic Sensitivity

The diagnostic sensitivity of real time (RT-) PCR assays is mainly dependent on the DNA/RNA extraction method used to isolate DNA and RNA from various biological specimens. DNA/RNA extraction reagents are not part of the gerbion real time (RT-) PCR kits. gerbion real time (RT-) PCR kits include an extraction control and guidelines for the validation criteria of the extraction control in each reaction. The extraction control indicates inhibition of the real time (RT-) PCR and/or inefficient nucleic acid extraction. It cannot be used as a calibrator.

Therefore, gerbion guarantees the analytical sensitivities and specificities of the real time (RT-) PCR kits, performed with eluted DNA and RNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. gerbion does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of gerbion real time (RT-) PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods. It is the responsibility of the user to

qualify the extraction methods used for DNA/RNA isolation from biological samples.

17 Abbreviations and Symbols

MRSA	Methicillin-resistant *Staphylococcus aureus*	REF	Catalog number
MS-MRSA	Methicillin-suceptible MRSA, mecA dropout mutant	Σ	Contains sufficient for <n> test
SCCmec/orfX	Junction for *S. aureus* DNA and SCCmec cassette	- 18°C	Upper limit of temperature
MSSA	Methicillin-suceptible *Staphylococcus aureus*		Manufacturer
MR-ConS	Methicillin-resistant coagulase negative *Staphylococcus*		Use by YYYY-MM-DD
mecA / mecC	Two varaints of the methicillin resistance gene	LOT	Batch code
DNA	Deoxyribonucleid Acid	CONT	Content
PCR	Polymerase Chain Reaction		Consult instructions for use
REACTION MIX	Reaction Mix	IVD	*In vitro* diagnostic medical device
CONTROL +	Positive Control	CE	European Conformity
CONTROL —	Negative Control		
CONTROL DNA IPC	Control DNA (IPC)		

18 Literature

[1] Bundesgesundheitsbl 2014, 57, 696–732: Empfehlungen zur Prävention und Kontrolle von Methicillin-resistenten Staphylococcus aureus-Stämmen (MRSA) in medizinischen und pflegerischen Einrichtungen.

[2] Centers for Disease Control and Prevention: Methicillin-resistant Staphylococcus aureus. www.cdc.gov/mrsa. May 16, 2016.