MIKROGEN
DIAGNOSTIK

Instruction for Use

*alpha*Cube PVL-MRSA

For simultaneous qualitive *in vitro* detection of Methicillin Resistant Staphylococcus aureus (MRSA) DNA and the differentiation of Community-acquired (CA) and Hospital-acquired (HA) MRSA extracted from biological specimens.

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1 Intended Use

The Kit is designed for the qualitative detection of the nucleic acid of Methicillin resistant Staphylococcus aureus (MRSA) and the differentiation of Community-acquired (CA) and Hospital-acquired (HA) MRSA in eluates from biological specimens. The assay is an in vitro diagnostic medical device and intended to be used by professional users in a laboratory environment. It can be performed manually or using an automated platform. The assay serves as an aid in the diagnosis, screening and monitoring of Methicillin resistant Staphylococcus aureus (MRSA).

2 Background 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 anitbiotics. 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. In the recent years also MRSA infections associated with livestock (livestock-associated MRSA or LA-MRSA) emerged, especially with pig farmers.

Since the mid-1990s the number of infections in the population increased with no previously history of medical facilitiy contact. This increase in infections in the population is caused by Staphylococcus aureus strains that carry the virulence factor Panton-Valentine leukocidin. Infections tend to occur in healthy younger people. PVL can be produced by methicillin-sensitive MSSA as well as MRSA. MRSA strains that carry the virulence factor PVL are called CA-MRSA. Panton-Valentine leukocidin (PVL) is a bicomponent, poreforming cytotoxin. The cytotoxin of PVL lyses macrophages as well as neutrophil granulocytes and contributes to tissue necrosis. The clinical manifestion of PVL-positive Staphylococcus aureus strains are skin and soft tissue infections, particularly recurrent invasive abscesses. Rarely necrotizing pneumonia develops with a mortality rate of up to 75%. Risk groups for transmission CA-MRSA or PVL-MSSA are for example families, persons performing close contact sports, persons from educational settings, prisoners and military personnel.

3 Principle of the Test

*alpha*Cube PVL-MRSA contains specific primers and dual-labeled probes for the amplification and detection of MRSA DNA in biological specimens. The PCR targets the SCCmec/orfX junction and allows for the detection of MRSA in biological samples, even those containing Coagulase-Negative Staphylococci. Furthermore, the real time PCR Kit allows the detection of the methicillin resistance gene mecA/mecC, to eliminate false positive results through dropout mutants. Additionally, the gene of PVL is detected.

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 SCCmec/orfX 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. The fluorescence of PVL-specific probes is detected in the ROX channel.
Furthermore, *alpha*Cube PVL-MRSA contains a Control DNA, which is added during DNA extraction and detected in the same reaction by a differently 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. The fluorescence of the Control DNA is measured in the HEX-channel.

4 Package Contents

The reagents supplied are sufficient for 96 reactions.

Table 1: Components of alphaCube PVL-MRSA.

Label	Lid Colour	Content
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. *alpha*Clean Pure RNA/DNA or *alpha*Clean Mag RNA/DNA)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- If using LightCycler® 480 II (Roche) alphaCube Colour Compensation (831009) is required.
- Optical PCR reaction tubes with lid
- Optional: Liquid handling system for automation

6 Transport, Storage and Stability

*alpha*Cube PVL-MRSA 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 Warning 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 mix components of different lots.

8 Sample Material

Starting material for diarellaPVL-MRSA real time PCR is DNA isolated from biological specimens. By the nature of the pathogens, sample material like nasal swabs or skin swabs is commonly used.

9 Sample Preparation

Purified DNA is suitable for downstream processing in real time PCR. For the extraction and purification of DNA from various biological materials, commercial kits are available. The operator needs to evaluate the suitability of respective DNA extraction kit. Commercial kits for DNA isolation such as the following are recommended:

- *alpha*Clean Pure RNA/DNA
- *alpha*Clean Mag RNA/DNA

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 of the respective nucleic acid extraction kit.

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 step of the extraction kit.

11 Real time PCR

11.1 Important Points Before Starting:

- Please pay attention to the chapter ‚Important Notes'.
- 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 (Control DNA was added during DNA extraction)

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 / 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 / optical PCR reaction plate (Table 3).
- Close the optical PCR reaction tubes / 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 the thermal profile in the table shown below.

Table 4: 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			
Denaturation	10 sec	95°C	45
Annealing and Extension	40 sec Aquisition at the end of this step	60°C	

The real time PCR thermal profile mentioned represents the universal settings for *alpha*Cube real time PCR and real time RT-PCR kits. Therefore, different kits can be used in the same run. For *alpha*Cube real time PCR kits used for amplification of DNA, the reverse transcription can be omitted. Dependent on the real time PCR instrument used, further instrument settings have to be adjusted according to the table below.

Table 5: Overview of the instrument settings required for *alpha*Cube PVL-MRSA.

Real time PCR Instrument	**Parameter Reaction Mix**	**Detection Channel**	**Notes**		
LightCycler 480II			*alpha*Cube 480 Colour Compensation (831009) required		
			Melt Factor	Quant Factor	Max Integration Time (sec)
	SCCmec/orfX	FAM (465-510)	1	10	1
	Control DNA	HEX (533-580)	1	10	2
	PVL	ROX (533-610)	1	10	2
	mecA/mec	Cy5 (618-660)	1	10	3
Stratagene Mx3000P / Mx3005P Agilent Aria Mx	SCCmec/orfX	FAM	Gain 8		Reference Dye: None
	Control DNA	HEX	Gain 1		
	PVL	ROX	Gain 1		
	mecA/mecC	Cy5	Gain 4		
ABI 7500 QuantStudio 5 CFX 96 CFX Opus 96	SCCmec/orfX	FAM	Option Reference Dye ROX: NO		
	Control DNA	JOE			
	PVL	ROX			
	mecA/mecC	Cy5			
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	SCCmec/orfX	Green	Gain 5		
	Control DNA	Yellow	Gain 5		
	PVL	Orange	Gain 5		
	mecA/mecC	Red	Gain 5		
mic qPCR Cycler	SCCmec/orfX	Green	Gain 8		
	Control DNA	Yellow	Gain 10		
	PVL	Orange	Gain 10		
	mecA/mecC	Red	Gain 10		

12 Data Analysis

Following results can occur:

Table 6: Interpretation of results

FAM SCCmec/ orfX	**ROX** PVL	**Cy5** mecA/ mecC	**HEX** Control DNA	**MRSA**	
+	+	+	positive or negative[1]	**positive**	Community-acquired MRSA (CA-MRSA, PVL-positive). The result for the Control DNA is irrelevant
+	-	+	positive or negative[1]	**positive**	Hospital-acquired MRSA (HA-MRSA, PVL-negative). The result for the Control DNA is irrelevant
+	+	-	positive or negative[1]	negative	CA-MSSA (methicillin sensitive). The result for the Control DNA is irrelevant
+	-	-	positive or negative[1]	negative	MSSA. The result for the Control DNA is irrelevant
-	+	+	positive or negative[1]	negative	CA-MSSA and MR-ConS. The result for the Control DNA is irrelevant
-	+	-	positive or negative[1]	negative	CA-MSSA. The result for the Control DNA is irrelevant
-	-	+	positive or negative[1]	negative	MR-ConS. The result for the Control DNA is irrelevant
-	-	-	≤ 34 [2]	negative	MRSA negative
-	-	-	> 34 [2]/ negative	?	Not interpretable

[1] A strong positive signal in the FAM, ROX or the Cy5 can inhibit the IPC. In such cases the result for the Control DNA can be neglected.
[2] In case of high C_T values, the IPC should be compared to the water extraction control as described in the chapter 'Assay validation'.

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 alphaClean Mag RNA/DNA. The Control DNA (IPC) must show a positive (i.e. exponential) amplification curve.
The IPC must fall below a C_T of 34. If the IPC 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.
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 Instructions 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 alphaCube PVL-MRSA 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.

No fluorescence signal in the specific channels of the Positive Control	
The selected channel for analysis does not comply with the protocol	Select the channel according to chapter ‚Instrument Settings‘.
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 chapter ‚Instrument Settings‘.

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 chapter ‚Transport, Storage and Stability‘.
Weak or no signal of the Control DNA and simultaneous absence of a signal in the specific channels	
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. An additional centrifugation step at high speed is recommended before elution of the DNA.
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 chapter ‚Transport, Storage and Stability‘.
Detection of a fluorescence signal in the specific channels 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.

16 Kit Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) of *alpha*Cube PVL-MRSA real time PCR was determined using serial dilutions of synthetic target DNA-sequences on a LightCycler 480 II real time PCR instrument. The LoD of *alpha*Cube PVL-MRSA real time PCR for MRSA is at least 2.5 copies per µl each.

16.2 Analytical Specificity

The specificity of the *alpha*Cube PVL-MRSA real time PCR was evaluated additionally with different other relevant viruses and bacteria found in clinical samples.
The *alpha*Cube PVL-MRSA real time PCR showed a positive result for the sample containing MRSA, whereas samples containing other pathogens were reliably tested negative. The results are shown in table 8 and 9.

Table 7: Bacterial and viral pathogens tested for the determination of the analytical specificity of the *alpha*Cube PVL-MRSA real time PCR Kit.

Strain	FAM	ROX	Cy5	MRSA
Borrelia burgdorferi	negative	negative	negative	negative
Coxsackievirus A9	negative	negative	negative	negative
Coxsackievirus B3	negative	negative	negative	negative
Cytomegalievirus	negative	negative	negative	negative
Enterococcus faecalis	negative	negative	negative	negative
Escherichia coli	negative	negative	negative	negative
Herpes simplex Virus 1	negative	negative	negative	negative
Herpes simplex Virus 2	negative	negative	negative	negative
Influenza A Virus H1N1	negative	negative	negative	negative
Influenza B Virus	negative	negative	negative	negative
Klebsiella spp.	negative	negative	negative	negative
Legionella pneumophila SG1	negative	negative	negative	negative
Legionella pneumophila SG2	negative	negative	negative	negative
Mycobacterium tuberculosis	negative	negative	negative	negative
Pneumocystis jirovecii	negative	negative	negative	negative
Pseudomonas aeruginosa	negative	negative	negative	negative
Respiratory syncitial Virus A	negative	negative	negative	negative
Respiratory syncitial Virus B	negative	negative	negative	negative
SARS-CoV-2	negative	negative	negative	negative

Strain	FAM	ROX	Cy5	MRSA
Staphylococcus areus (MSSA)	negative	negative	negative	negative
Staphylococcus intermedius	negative	negative	negative	negative
Staphylococcus sciuri	negative	negative	negative	negative
Streptococccus ueberis	negative	negative	negative	negative
Streptococcus agalactiae	negative	negative	negative	negative
Streptococcus dysgalactiae	negative	negative	negative	negative
Tick borne encephalitis Virus	negative	negative	negative	negative
Varizella-Zoster virus	negative	negative	negative	negative

Table 8: Ring trial samples tested for the determination of the analytical specificity of the *alpha*Cube PVL-MRSA real time PCR Kit.

Sample	expected result	*alpha*Cube PVL-MRSA
2025391 MRSA (S. aureus, PVL-neg, pSA442 neg)	positive	positive
2025392 CoNS oxaS	negative	negative
2025393 cMRSA (S. aureus, oxaR, PVL-pos)	positive	positive
2025394 cMRSA (S. aureus, oxaR, PVL-pos)	positive	positive
2125391 MRSA (S. aureus, oxaR, PVL-neg)	positive	positive
2125392 Escherichia coli K12	negative	negative
2125393 MRSA (S. aureus, oxaR, PVL-neg)	positive	positive
2125394 cMSSA (S. aureus, oxaS, PVL-pos)	negative	negative
2225391 MSSA + CoNS (S. aureus, S. epidermidis oxaR, PVL-neg)	negative	negative
2225392 Escherichia coli K12	negative	negative
2225393 MRSA (S. aureus, oxaR, PVL-neg)	positive	positive
2225394 cMRSA (S. aureus, oxaR, PVL-pos)	positive	positive
MRSADNA24S-01 MRSA mecC	positive	positive
MRSADNA24S-02 MRSA N315	positive	positive
MRSADNA24S-03 MRSA N315 + MRCoNS 634	positive	positive
MRSADNA24S-04 MRSA N315	positive	positive
MRSADNA24S-05 MSSA 29213 + MRCoNS 634	negative	negative

Sample	expected result	*alpha*Cube PVL-MRSA
MRSADNA24S-06 MRSA ST398	positive	positive
MRSADNA24S-07 MRSA ST398	positive	positive
MRSADNA24S-08 MRSA N315 + MSSA 29213	positive	positive
MRSADNA24S-09 Negative	negative	negative
MRSADNA24S-10 MRSA N315	positive	positive

16.3 Linear Range

The linear range of the *alpha*Cube PVL-MRSA was evaluated by analyzing logarithmic dilution series of synthetic DNA fragments.

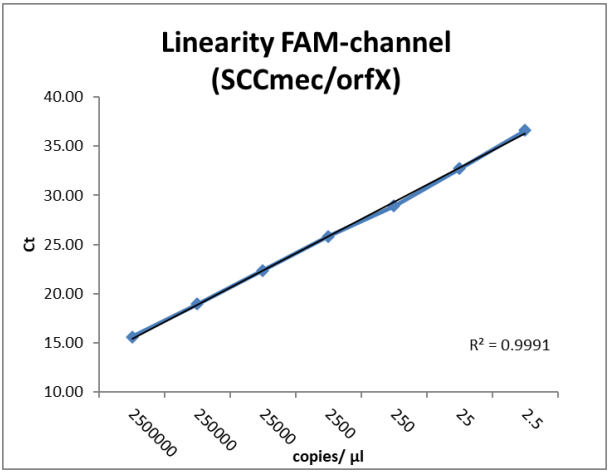

Figure 1: Determination of the linear range of the alphaCube PVL-MRSA real time PCR Kit for the SCCmec/orfX junction in the FAM channel.

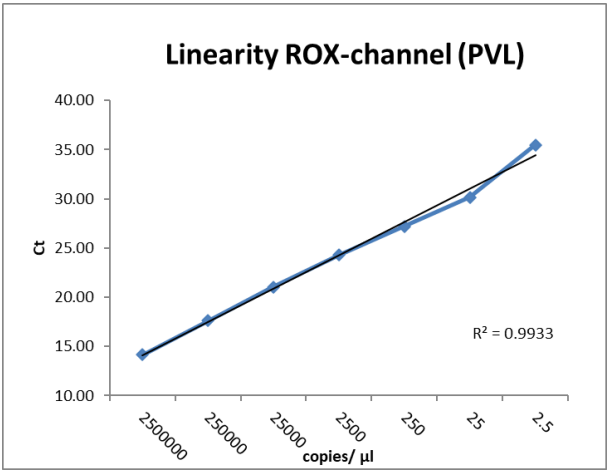

Figure 2: Determination of the linear range of the alphaCube PVL-MRSA real time PCR Kit for PVL in the ROX channel.

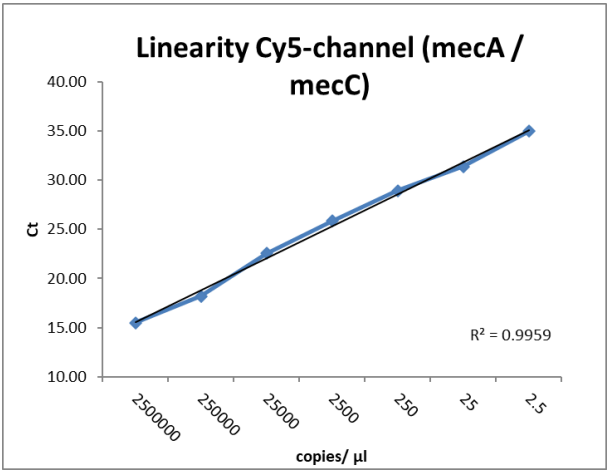

Figure 3: Determination of the linear range of the alphaCube PVL-MRSA real time PCR Kit for mecA / mecC gene in the Cy5 channel.

16.4 Precision

The precision of the *alpha*Cube PVL-MRSA was determined as intra-assay variability, inter-assay variabilitity 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 MRSA SCCmec/orfX, MRSA mecA/mecC and PVL specific DNA and on the threshold cycle of the Control-DNA.

Table 9: Precision of alphaCube PVL-MRSA

SCCmec/orfX	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	250	0.43	1.49
Inter-Assay-Variability	250	0.30	1.06
Inter-Lot Variability	250	0.64	2.25

mecA/mecC	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	250	1.22	4.20
Inter-Assay-Variability	250	0.26	0.87
Inter-Lot Variability	250	0.12	0.41

PVL	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	250	0.10	0.36
Inter-Assay-Variability	250	0.15	0.52
Inter-Lot Variability	250	0.08	0.26

Control DNA	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	1250	0.71	2.44
Inter-Assay-Variability	1250	0.22	0.74
Inter-Lot Variability	1250	0.32	1.09

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
SCCmec/orfX	Junction for *S. aureus* DNA and SCCmec cassette	Σ	Contains sufficient for <n> test
MSSA	Methicillin-suceptible *Staphylococcus aureus*	-18°C	Upper limit of temperature
MR-ConS	Methicillin-resistant coagulase negative *Staphylococcus*		Manufacturer
mecA / mecC	Two varaints of the methicillin resistance gene		Use by YYYY-MM-DD
PVL	Panton-Valentine-Leucocidine	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 IC	Control DNA		

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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.

[3] Epidemiologisches Bulletin, https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2018/Ausgaben/05_18.pdf: Eigenschaften, Häufigkeit und Verbreitung von MRSA in Deutschland, update 2015/2016.

19 Change History

Version	Change	Date
1.2	Change in volume for the Real time PCR set up, therefore following chapters were updated: 4 - Package Contents, 11 - Real time PCR, 16 - Kit Performance Updating of chapters: 1- Intended Use, 10 – Control DNA, 12 - Data Analysis (naming CA-MS-MRSA to CA-MSSA and MS-MRSA to MSSA) 13 - Assay Validation, 7 - Warning and Precautions, 8 – Sample Material, 11.3 - Instrument Settings Addition chapter 19	2025-01-29