Manual

MutaPLEX® PVL-MRSA real time PCR kit

Test for the qualitative in vitro detection of methicillin-resistant Staphylococcus aureus (MRSA) DNA and the differentiation of community-acquired (CA) and hospital-acquired (HA) MRSA

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Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

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1. INTENDED USE

The MutaPLEX® PVL-MRSA real time PCR is a multiplex real time PCR for the qualitative detection and differentiation of DNA of methicillin-resistant *Staphylococcus aureus* (MRSA, PVL-negative) and methicillin-sensitive *Staphylococcus aureus* (drop-out mutant of MRSA, MS-MRSA, PVL-negative) or methicillin-resistant, coagulase-negative *Staphylococci* (MR-ConS, PVL-negative) and the qualitative detection and differentiation of DNA of methicillin-resistant, community-associated *Staphylococcus aureus* (CA-MRSA, PVL-positive) and methicillin-sensitive, community-associated *Staphylococcus aureus* (CA-MSSA, PVL-positive) or methicillin-resistant coagulase-negative *Staphylococci* (MR-ConS, PVL-positive).

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 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 facility 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

The MutaPLEX® PVL-MRSA real time PCR kit contains specific primers and dual-labelled probes for the amplification and detection of MRSA DNA in clinical specimens. The PCR targets the orfX/SSCmec junction and allows for the detection of MRSA in clinical samples, even those containing coagulase-negative *Staphylococci*. Furthermore, MutaPLEX® PVL-MRSA 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. The fluorescence of PVL-specific probes is detected in the ROX channel.

Furthermore, MutaPLEX® PVL-MRSA real time PCR kit contains a control DNA, which is added during DNA extraction and detected in the same reaction by a differently labelled probe.

The control DNA allows the detection of PCR inhibition and acts as control for the isolation of the nucleic acid from the clinical 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 the MutaPLEX® PVL-MRSA real time PCR kit .

Label	Lid Colour	Content
Reaction Mix	yellow	1 x 1536 µl
Positive control	red	1 x 100 µl
Negative control	green	1 x 100 µl
Control DNA	colourless	1 x 480 µl

5. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- DNA purification kit (e. g. MutaCLEAN® Universal RNA/DNA, KG1038. or the magnet particle-based system MutaCLEAN® Mag RNA/DNA, KG1023)
- PCR grade water

- Sterile microtubes
- Pipets (adjustable volume)
- DNase/RNase-free disposable pipette tips with aerosol barriers
- Table centrifuge
- Vortex mixer
- Real time PCR instrument
- If using a Roche LightCycler® 480 II device, the Colour Compensation kit MutaPLEX® CC-1 (KG19-5-CC) is required.
- Optical PCR reaction tubes or optical PCR reaction plates with optical foil
- Optional: Liquid handling system for automation

  * Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696). which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6. TRANSPORT. STORAGE AND STABILITY

The MutaPLEX® PVL-MRSA real time PCR kit is shipped on dry ice. All components must be stored at maximum - 20 °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.
- 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 pipet 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.

8. 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 for DNA isolation such as MutaCLEAN® Universal RNA/DNA (KG1038) or the magnet particle-based system MutaCLEAN® Mag RNA/DNA (KG1023) are recommended. The operator needs to evaluate the suitability of respective DNA 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 chapter 11 "Control DNA".

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

9. CONTROL DNA

A control DNA is supplied and can be used as extraction control or only as inhibition control. This allows the user to control the DNA isolation procedure and to check for possible real time PCR inhibition.

a) Control DNA used as extraction control

MutaPLEX® PVL-MRSA control DNA is added to the DNA extraction.

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

The control DNA must be added to the lysis buffer of the extraction kit.

b) Control DNA used as internal control of the real time PCR

If only inhibition will be checked, please follow protocol B.

10. REAL TIME PCR

10.1 Important points before starting

- Please pay attention to 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, thouroughly mixed, and centrifuged very briefly.
- We recommend to keep reagents and samples at 2 – 8 °C (e. g. on ice or a cooling block) at all times.

10.2 Procedure

If the control DNA is used to control both, the real time PCR and the DNA isolation procedure, please follow protocol A. If the control DNA is solely used to detect possible inhibition of the real time PCR, please follow protocol B.

Protocol A

The control DNA was added during DNA extraction (see chapter "Control DNA"). In this case. prepare the master mix according to table 2.

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
16 µl Reaction Mix	16 µl x (N+1)

Protocol B

The control DNA is used for the control of the real time PCR only (see chapter 11 "Control DNA"). In this case, prepare the master mix according to table 3.

The master mix contains all of the components needed for real 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 3: Preparation of the master mix (control DNA is added directly to the master mix)

Volume per reaction	Volume master mix
16 µl Reaction Mix	16 µl x (N+1)
0.5 µl Control DNA *	0.5 µl x (N+1)*

*The increase in volume caused by adding the control DNA is not taken into account when preparing the PCR assay.

Protocol A and B: real time PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument or take an optical PCR reaction plate.
- Pipet **16 µl** of the master mix into each optical PCR reaction tube / optical PCR reaction plate.
- Add **4 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 4).
- Close the optical PCR reaction tubes / optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the real time PCR

Component	Volume
Master mix	16.0 µl
Sample	4.0 µl
Total volume	20.0 µl

10.3 Instrument settings

For the real time PCR use the thermal profile shown in table 5.

Table 5: real time PCR thermal profile

Description	Time	Temperature	No of cycles
Reverse transcription	10 min	45 °C	1
Initial Denaturation	5 min	95 °C	1
Amplification of DNA			45
Denaturation	10 s	95 °C	
Annealing and extension	40 s	60 °C	
	Acquisition at the end of this step		

The real time PCR thermal profile mentioned represents the universal settings for Immundiagnostik real time PCR and real time RT-PCR kits. Therefore, different kits can be used in the same run. For Immundiagnostik real time PCR kits used for amplification of DNA, the reverse transcription can be omitted. 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 MutaPLEX® PVL-MRSA real time PCR.

Real time PCR Instrument	Parameter	Detection Channel	Notes		
LightCycler 480II			Colour compensation kit MutaPLEX® CC-1 (KG19-5-CC) required		
			Melt factor	**Quant factor**	**Max integration time (s)**
	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 Aria MX	SCCmec/orfX	FAM	Gain 8	Reference dye: none	
	Control DNA	HEX	Gain 1		
	PVL	ROX	Gain 1		
	mecA/mec	Cy5	Gain 4		

Real time PCR Instrument	Parameter	Detection Channel	Notes	
ABI 7500 CFX96	SCCmec/orfX Control DNA PVL mecA/mec	FAM HEX ROX Cy5	Option reference dye ROX: NO	
Rotor-Gene Q. Rotor-Gene 3000 Rotor-Gene 6000	SCCmec/orfX Control DNA PVL mecA/mec	Green Yellow Orange Red	Gain 5 Gain 5 Gain 5 Gain 5	
mic Q-PCR cycler	SCCmec/orfX Control DNA PVL mecA/mec	Green Yellow Orange Red	Gain 8 Gain 10 Gain 10 Gain 10	

11. DATA ANALYSIS

The following results can occur:

FAM SCCmec/ orfX	ROX PVL	Cy5 mecA/ mecC	HEX Control DNA	MRSA	Interpretation
+	+	+	+ / –*	**positive**	**Community-acquired MRSA** (CA-MRSA. PVL-positive). The result for the control DNA is irrelevant
+	–	+	+ / –*	**positive**	**Hospital-acquired MRSA** (HA-MRSA. PVL-negative). The result for the control DNA is irrelevant
+	+	–	+ / –*	negative	CA-MS-MRSA (methicillin sensitive). The result for the control DNA is irrelevant
+	–	–	+ / –*	negative	HA-MS-MRSA. The result for the control DNA is irrelevant

FAM SCCmec/ orfX	ROX PVL	Cy5 mecA/ mecC	HEX Control DNA	MRSA	Interpretation
–	+	+	+ / –*	negative	CA-MSSA and MR-ConS. The result for the control DNA is irrelevant
–	+	–	+ / –*	negative	CA-MSSA. The result for the control DNA is irrelevant
–	–	+	+ / –*	negative	MR-ConS. The result for the control DNA is irrelevant
–	–	–	≤ 34**	negative	MRSA negative
–	–	–	> 34**/ negative	?	Not interpretable

* A strong positive signal in the FAM. Cy5 and/or ROX channel can inhibit the amplification of the Control DNA. In such cases, the result for the control DNA can be neglected.

** Depending on the PCR instrument and/or the chosen extraction method, the C_T values might be shifted. The water control can be used as reference. If the HEX C_T value of a sample differs a lot from the water control, partial inhibition has occurred, leading to false negative results in case of weak positive samples.

Figure 1 and figure 2 show examples for positive and negative real time PCR results.

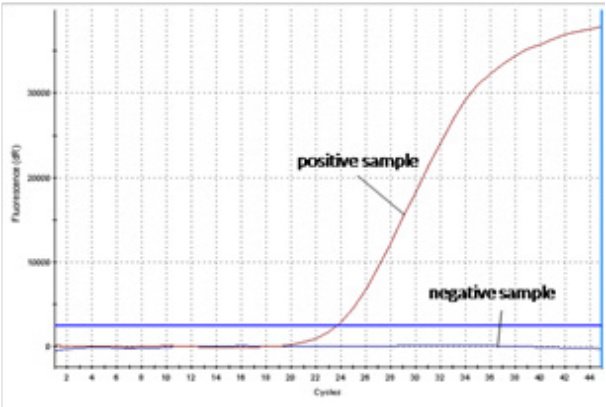


Figure 1: The positive sample shows amplification signal in the specific channel (FAM/Cy5/ROX), whereas no fluorescence signal is detected in the negative sample.

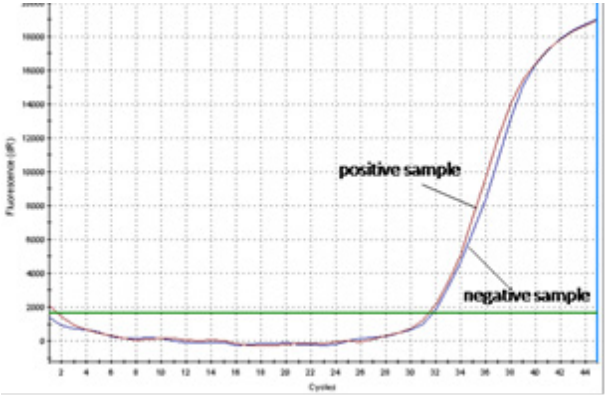


Figure 2: The positive sample as well as the negative sample show a signal in the control DNA-specific HEX channel. The amplification signal of the control DNA in the negative sample shows that the missing signal in the specific channel (FAM/Cy5/ROX) is not due to PCR inhibition or failure of DNA isolation, but that the sample is a true negative.

12. ASSAY VALIDATION

Set a threshold as follows:

Negative controls

All negative controls should be below the threshold. If there is a potential contamination (appearance of a curve in the negative control or a cluster of curves in specimens at high C_T – for example above 36), results obtained are not interpretable and the whole run (including extraction) has to be repeated.

Positive controls

All the positive controls must show a positive (i. e. exponential) amplification curve. The positive controls must fall below a C_T of 30.

Internal controls

All internal controls must show a positive (i. e. exponential) amplification curve. The internal control must fall below a C_T of ≤34. If the internal control is above C_T 34. this points to a purification problem or a strong positive sample that can inhibit the internal control. In the latter case, the assay is valid. If a water control run is performed, the internal control must fall below a C_T of ≤34.

13. LIMITATIONS

- 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 MutaPLEX® PVL-MRSA real time PCR kit need to be interpreted in consideration of all clinical and laboratory findings.

14. 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@immundiagnostik.com.

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 "Control DNA").

real time PCR inhibited

Make sure that you use an appropriate isolation method (see "Sample preparation") and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffer of the isolation kit has 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 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 occurred 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.

15. KIT PERFORMANCE

15.1 Analytical sensitivity

The limit of detection (LoD) of MutaPLEX® PVL-MRSA real time PCR kit was determined using serial dilutions of synthetic target DNA sequences in a Stratagene Mx3005 real time PCR instrument. The LoD of MutaPLEX® PVL-MRSA real time PCR kit is at least 10 copies per reaction each.

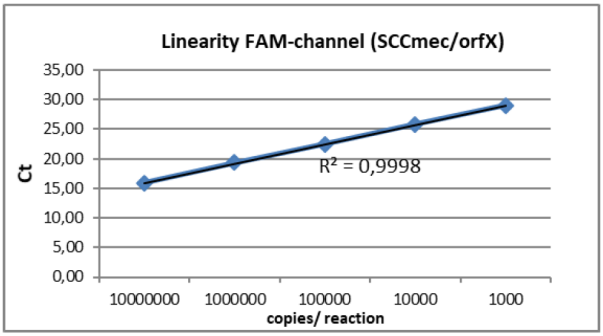
Sample	Concentration copies / reaction	C_T-value	mean C_T
		FAM channel	
SCCmec/ orfX	100,000,000	12.10 11.93 12.28	12.10
SCCmec/ orfX	10,000,000	15.86 16.00 15.76	15.87
SCCmec/ orfX	1,000,000	19.40 19.26 19.32	19.33
SCCmec/ orfX	100,000	22.54 22.24 22.55	22.44
SCCmec/ orfX	10,000	25.87 25.73 25.87	25.82
SCCmec/ orfX	1,000	28.60 29.14 29.22	28.99
SCCmec/ orfX	100	29.88 30.37 29.52	29.92
SCCmec/ orfX	10	31.31 31.90 32.49	31.90
SCCmec/ orfX	1	32.43 32.16 31.76	32.12

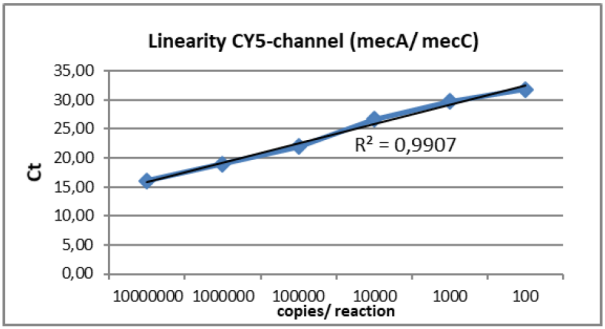
Sample	Concentration copies / reaction	C_T-value	mean C_T
		Cy5 channel	
mecA/mecC	100,000,000	10.84 12.46 12.62	11.97
mecA/mecC	10,000,000	15.41 16.01 16.63	16.02
mecA/mecC	1,000,000	19.63 18.76 18.50	18.96
mecA/mecC	100,000	22.87 21.34 21.67	21.96
mecA/mecC	10,000	26.63 26.79 26.52	26.65
mecA/mecC	1,000	29.72 29.91 29.50	29.71
mecA/mecC	100	32.40 32.19 30.81	31.80
mecA/mecC	10	31.54 34.07 34.61	33.41
mecA/mecC	1	45.00 45.00 39.83	43.28

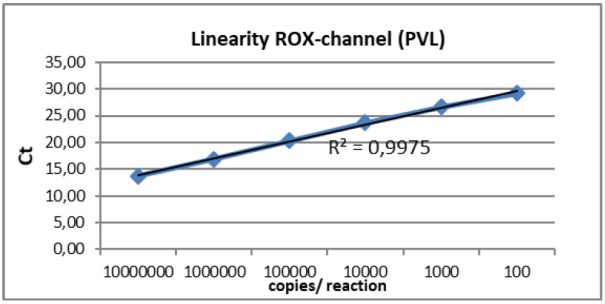
Sample	Concentration copies / reaction	C_T-value	mean C_T
		ROX channel	
PVL	100,000,000	10.55 10.08 10.00	10.21
PVL	10,000,000	13.71 13.67 13.67	13.68
PVL	1,000,000	16.94 17.03 16.82	16.93
PVL	100,000	20.24 20.25 20.38	20.29
PVL	10,000	23.68 23.69 23.72	23.70
PVL	1,000	26.66 26.60 26.69	26.65
PVL	100	29.14 29.21 29.18	29.18
PVL	10	30.13 30.04 30.31	30.16
PVL	1	30.76 30.65 30.83	30.75

15.2 Linear range

The linear range of the MutaPLEX® PVL-MRSA real time PCR was evaluated by analysing logarithmic dilution series of synthetic DNA fragments.







15.3 Analytical specificity

The specificity of the MutaPLEX® PVL-MRSA real time PCR kit was evaluated additionally with different other relevant viruses and bacteria found in clinical samples.

Results

The MutaPLEX® 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 7 and table 8.

Table 7: Bacterial and viral pathogens tested for the determination of the analytical specificity of the MutaPLEX® PVL-MRSA real time PCR kit.

Strain	FAM	ROX	Cy5	MRSA
Streptococcus agalactiae	negative	negative	negative	negative
Coxsackievirus Strain P.B.	negative	negative	negative	negative
Coxsackievirus Strain B.S.	negative	negative	negative	negative
Herpes simplex virus	negative	negative	negative	negative
Borrelia burgdorferi	negative	negative	negative	negative
S. uberis	negative	negative	negative	negative
Streptococcus dysagalactiae	negative	negative	negative	negative
Staphylococcus intermedius	negative	negative	negative	negative
Pseudomonas aeruginosa	negative	negative	negative	negative
Staphylococcus sciuri	negative	negative	negative	negative
Legionella pneumophila Serogroup 1	negative	negative	negative	negative

Table 8: Ring trial samples tested for the determination of the analytical specificity of the MutaPLEX® PVL-MRSA real time PCR kit.

Strain	Expected result	MutaPLEX® PVL-MRSA Result
1815391 cMRSA (S. aureus, oxaR, PVL-pos, spa:t 008	positive	positive
1815392 MRSA (S. aureus, oxaR, PVL-neg)	positive	positive
1815393 Escherichia coli K12	negative	negative
1815394 cMSSA + CoNS (S. aureus, S. epidermidis oxaR, PVL-pos)	negative	negative

Strain	Expected result	MutaPLEX® PVL-MRSA Result
1715391 MRSA (S. aureus, oxaR, PVL-neg)	positive	positive
1715392 MSSA (SCCmec pos, mecA neg) (S. aureus, oxaS, PVL-neg. spa:t 310)	negative	negative
1715394 MRSA (S. aureus, oxaR, PVL-neg)	positive	positive
1615391 MSSA + CoNS (S. aureus, S. epidermidis oxaR, PVL-neg)	negative	negative
1615392 cMRSA (S. aureus, oxaR, PVL-pos, spa:t 310)	positive	positive
1615393 CoNS (S. epidermidis, oxaS)	negative	negative
1615394 cMRSA (S. aureus, oxaR, PVL-pos, spa:t 008	positive	positive
1025391 MRSA + CoNS (S. aureus, CoNS, oxaR, PVL-neg)	positive	positive
1025392 MSSA + CoNS (S. aureus, CoNS, oxaS) "mecA dropout mutant"	negative	negative
1025393 cMRSA + CoNS (S. aureus, CoNS, oxaR, PVL-pos)	positive	positive
1025394 CoNS (oxaS)	negative	negative
1015391 MRSA ("Züricher Drogenstamm"; CHE 482) SCCmec PCRs (S. aureus, oxaR, PVL-neg)	positive	positive
1015393 MSSA + CoNS (S. aureus, CoNS, oxaR)	negative	negative
92901 cMSSA + CoNS (S. aureus, S. epidermidis oxaS, PVL-pos)	negative	negative
92902 cMRSA spa:t 310 (S. aureus, oxaR, PVL-pos)	positive	positive
92904 CoNS (S. epidermidis, oxaR)	negative	negative
91901 cMRSA spa:t 657 (S. aureus, oxaR, PVL-pos)	positive	positive
91902 MSSA + CoNS (S. epidermidis, oxaR)	negative	negative

Strain	Expected result	MutaPLEX® PVL-MRSA Result
1515391 MRSA SCCmec Typ V (S. aureus, oxaR, PVL-neg)	positive	positive
1515394 MSSA + CoNS (S. aureus, S. epidermidis oxaR, PVL-neg)	negative	negative
12-02 MRSA N315 (Core)	positive	positive
12-03 MRSA N315 (Core)	positive	positive
12-04 MSSA ATCC 29213 (Core)	negative	negative
12-05 MRSA ST398	positive	positive
12-06 MRSA N315	positive	positive
12-07 MRSA N315 (Core)	positive	positive
12-08 MSSA 29213 + MRCoNS 634 (Core)	negative	negative
12-09 MRSA N315 (Core)	positive	positive
12-10 MRSA Negative MHB (Core)	negative	negative
12-11 MRSA "mecC"	positive	positive
12-12 E. coli ATCC 35218 (Core)	negative	negative

15.4 Precision

The precision of the MutaPLEX® PVL-MRSA real time PCR 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 MRSA SCCmec/orfX, MRSA mecA/mecC and PVL specific DNA and on the threshold cycle of the control DNA.

Table 9: Precision of MutaPLEX® PVL-MRSA real time PCR.

SCCmec/orfX	copies/rxn	Standard deviation	Coefficient of variation [%]
Intra-Assay variability	1.000	0.43	1.49
Inter-Assay variability	1.000	0.30	1.06
Inter-Lot variability	1.000	0.64	2.25

mecA/mecC	copies/rxn	Standard deviation	Coefficient of variation [%]
Intra-Assay variability	1.000	1.22	4.20
Inter-Assay variability	1.000	0.26	0.87
Inter-Lot variability	1.000	0.12	0.41

PVL	copies/rxn	Standard deviation	Coefficient of variation [%]
Intra-Assay variability	100	0.10	0.36
Inter-Assay variability	100	0.15	0.52
Inter-Lot variability	100	0.08	0.26

Control DNA	copies/rxn	Standard deviation	Coefficient of variation [%]
Intra-Assay variability	100	0.71	2.44
Inter-Assay variability	100	0.22	0.74
Inter-Lot variability	100	0.32	1.09

16. ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleic Acid	PVL	Panton-Valentine-Leucocidine
C_T	Cycle threshold	CONTROL DNA | IC	Control DNA
MRSA	Methicillin-resistant *Staphylococcus aureus*	→REF	To be used with
MS-MRSA	Methicillin-susceptible MRSA. mecA dropout mutant	SCCmec/orfX	Junction for S. aureus DNA and SCCmec cassette
MSSA	Methicillin-susceptible *Staphylococcus aureus*	MR-ConS	Methicillin-resistant coagulase negative Staphylococcus
mecA / mecC	Two variants of the methicillin resistance gene		Manufacturer
PCR	Polymerase Chain Reaction	REF	Catalog number
CONTROL | –	Negative control		Contains sufficient for <n> test
CONTROL | +	Positive control		Upper limit of temperature
REACTION MIX	Reaction Mix		Use by
CONTENT	Content	LOT	Lot number
	Consult instructions for use	IVD	In vitro diagnostic medical device

17. LITERATURE

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Immundiagnostik AG

Stubenwald-Allee 8a
64625 Bensheim, Germany

Tel.: +49 6251 70190-0
Fax: +49 6251 70190-363

info@immundiagnostik.com
www.immundiagnostik.com