Instructions for Use

In-vitro-Diagnostic

IVD

The MRSA *direct* PCR is a real-time PCR test for *in vitro* detection and differentiation of DNA from methicillin-resistant *Staphylococcus aureus* **(MRSA**), vancomycin-resistant *Staphylococcus aureus* (**VRSA**) and Methicillin-susceptible *Staphylococcus aureus* (**MSSA**).

Ref.No.:
FBC131-Ax
FBC131-Bx
FBC131-Cx

The MRSA *direct* PCR test was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™. In general, Lab *direct* qPCR tests are compatible with other qPCR cyclers (e.g., MIC Cycler, AJ qTOWER, LightCycler® 96 or 480 I).

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1. Name of the Device

MRSA *direct* PCR

2. Intended Purpose

The MRSA *direct* PCR is a qualitative assay for *in vitro* examination of bacterial DNA in nasal/throat swabs, swabs from skin/wounds or culture from patients to provide information to aid to diagnose symptomatic or asymptomatic patients under suspicion of infection with methicillin-resistant *Staphylococcus aureus* (MRSA) and/or vancomycin-resistant *Staphylococcus aureus* (VRSA) as well as Methicillin-susceptible *Staphylococcus aureus* (MSSA).

The IVD medical device detects the DNA of the aforementioned pathogens by qualitative measurements based on qPCR and is intended for use in medical laboratories or health institutions by laboratory personnel specifically trained in qPCR and in vitro diagnostic techniques. It has to be used in combination with conventional qPCR cyclers for detection and analysis.

3. Pathogen Information

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant Staphylococcus aureus (MRSA) is a Gram-positive opportunistic pathogen that colonizes approximately 20–30 % of the human population and can cause a broad spectrum of diseases ranging from superficial skin and soft tissue infections to severe invasive infections such as bacteremia, pneumonia, endocarditis, and sepsis. MRSA emerged shortly after the clinical introduction of methicillin in the early 1960s and has since developed into one of the most clinically relevant antimicrobial-resistant pathogens worldwide. This is particularly evident in healthcare settings, where selective pressure caused by extensive antibiotic use promotes the persistence, adaptation, and spread of resistant strains. [1]

Epidemiological data demonstrate that MRSA occurrence is dynamic and influenced by factors such as geographical region, healthcare infrastructure, infection prevention strategies, and antibiotic usage patterns. Trends in MRSA incidence show considerable temporal variation and differ between epidemiological categories, including hospital-onset, healthcare-associated community-onset, and community-associated infections. Although decreases in MRSA incidence have been observed in certain healthcare systems, largely due to improved infection control measures, MRSA continues to represent a substantial burden and remains a key pathogen in global antimicrobial resistance surveillance programs. [2]

Staphylococci naturally colonise the skin and mucosa of the nose and oropharynx. They are divided into coagulase-positive (*S. aureus*) and coagulase-negative staphylococci (CoNS, such as *S. epidermidis*). An estimated 30% of the healthy population is colonised with *S. aureus* (asymptomatic carriers). MRSA is one of the most frequent pathogens of nosocomial infections worldwide (hospital-acquired MRSA or just HA-MRSA). In addition to HA-MRSA infections, there are also community-acquired MRSA (CA-MRSA) infections that are acquired outside of the hospital. [4]

The methicillin (oxacillin)-resistance of *S. aureus* is mediated by the penicillin-binding protein PBP2a, which is encoded by the chromosomal *mecA* gene. The *mecA* gene is localised on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec). To date, multiple SCCmec cassette types have been described, of which types I to V occur most frequently. [5] The SCCmec cassette type XI (SCCmec XI) contains another *mecA* homolog, also termed *mecC* or mecLGA251, and was first described in 2011. [6] The *mecC* gene has only a nucleotide homology of 70% with *mecA* and is not detectable using normal *mecA*-specific primers. The insertion site of SCCmec into the *orfX* gene of *S. aureus* (Mec right extremity junction, MREJ) is specific for MRSA and can be used to distinguish MRSA from commensal CoNS carrying SCCmec elements. [7]

In contrast to infections with MSSA (methicillin-sensitive *Staphylococcus aureus*), MRSA infections are associated with an elevated morbidity, mortality, extended hospital stays and greater treatment costs. Rapid MRSA screening allows for appropriate treatment and patient management to prevent the transmission and spread of MRSA. [8] Using conventional culturing methods, 48 to 72 hours are needed to detect MRSA, whereas real-time PCR tests allow early and fast MRSA screening on the date of hospital admission.

Methicillin-susceptible *Staphylococcus aureus* (MSSA)

Methicillin-susceptible *Staphylococcus aureus* (MSSA) strains lack the *mecA* or *mecC* resistance genes and therefore do not produce the altered penicillin-binding protein PBP2a that mediates methicillin resistance. [3][9] MSSA remains a major cause of both community-acquired and healthcare-associated infections, including skin and soft tissue infections, pneumonia, bloodstream infections, endocarditis, and osteomyelitis. [3][10] Although MSSA infections are generally associated with more treatment options and better clinical outcomes than MRSA infections, they can still cause severe invasive disease with significant morbidity and mortality. [9][10] In contrast to MRSA, MSSA isolates are usually susceptible to anti-staphylococcal β-lactam antibiotics such as oxacillin, flucloxacillin, or cefazolin, which are considered the preferred treatment options. [10] Nevertheless, the pathogenicity of MSSA remains largely comparable to that of MRSA because many virulence factors, including toxins and adhesion proteins, are shared between susceptible and resistant *S. aureus* strains. [3][9]

Vancomycin-resistant *Staphylococcus aureus* (VRSA)

Vancomycin-resistant *Staphylococcus aureus* (VRSA) represents a rare but clinically significant extension of antimicrobial resistance evolution. First reported in 2002, VRSA strains show resistance to glycopeptide antibiotics such as vancomycin, which are commonly used as last-line therapeutic agents for MRSA infections. Although only a limited number of VRSA cases have been documented globally, the emergence of these strains raises significant concerns due to the severely limited treatment options and the potential for horizontal gene transfer of resistance determinants, which may facilitate broader dissemination [6].

4. Testing Principle

The MRSA *direct* PCR test is a multiplex qPCR test for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) as well as Methicillin-susceptible *Staphylococcus aureus* (MSSA).

The assay can be performed either on purified nucleic acids obtained through a prior extraction procedure (cf. chapter 11) or directly on the sample material without prior isolation and purification (cf. chapter 12).

For workflows including nucleic acid extraction, a separate Internal Control (IC) is added to each sample prior to DNA extraction and serves as control for nucleic acid isolation from the biological specimen as well as for amplification.

For direct testing without isolation and purification, a separate solution (Solution B) is provided containing lysis enzymes to break down the cell walls. Solution B and Internal Control are directly added to the reaction mix. IC functions as an amplification control, only.

The nucleic acid eluate or direct sample material is added to the ready-to-use reaction solution that contains all reagents necessary for qPCR. The analysis can be performed on various qPCR cyclers.

Possible sample material from different sources (nasal/throat swabs, swabs from skin/wounds or culture) include:

- samples provided as dry swabs
- samples provided as Amies Agar gel swabs
- samples provided in Copan eSwabs™
- samples provided in Copan UTM-RT System (UTM-RT)
- samples provided in Yocon VTM/UTM
- samples provided in NaCl or PBS solution
- samples provided in Amies solution
- samples provided in or in IsoFlow Steath Fluid (Beckman Coulter)
- Tryptic Soy Broth (TSB)

The use of other solubilisation media may lead to false-negative or invalid results. Samples containing chaotropic salts, such as guanidinium thiocyanate or polihexanide at a concentration of 0.25 µg/mL, must not be used (see chapter 15/Limitations of the method).

The direct testing procedure for **dry swabs and Amis Agar gel swabs** is given in Chapter 12 as **procedure I**
The direct testing procedure for **swabs provided in transport solutions** is given in Chapter 12 as **procedure II**

The MRSA *direct* PCR test contains primer and probes specific for the targets listed in Table 1 and the Internal Control. The probes are each labelled with fluorescent reporter dyes and a second dye that serves as a quencher and suppresses the fluorescence signals of intact probes.

The analysis is performed by determining Ct (cycle threshold) values. In general, the Ct value describes the cycle in which the signal rises above a certain threshold for the first time. The more target copies are present in the sample, the lower the Ct value. Nevertheless, the device is a qualitative test, only.

Table 1: Analytes and gene regions of MRSA direct PCR

ANALYTE	GENE	REGION (NUCLEOTIDES)	KIT VARIANT
Methicillin-resistant *Staphylococcus aureus* (MRSA)	*orfX*	variable (depending on SCCmec/MREJ type*)	FBC131-Ax FBC131-Bx FBC131-Cx
	mecA	1 – 2457	
	mecC	1 – 1998	
	spa	1 – 1377	
Vancomycin-resistant *Staphylococcus aureus* (VRSA)	*vanA*	1 – 1232	FBC131-Ax FBC131-Bx
	spa	1 – 1377	
Methicillin-susceptible *Staphylococcus aureus* (MSSA)	*spa*	1 – 1377	FBC131-Ax FBC131-Bx FBC131-Cx

* I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV

(see chapter 16.1/Analytical Sensitivity (LoD) for further information on the most relevant types)

5. Package Content

Each kit contains the following vials which are sufficient for 96 reactions (see Table 2).

Table 2: Package content of MRSA direct PCR

MATERIAL	LID COLOUR	#VIALS; VOLUME	#RXNS.	COMMENT	ACTIVE INGREDIENT(S)
Solution A	green	1x; 1050 µL	96	Reaction mix	Polymerase (approx. 0.2 U/µl); Primers and probes (approx. 0.2 µM)
Solution B	yellow	1x; 23 µL	96	Lysis mix	Lysis enzymes
Internal Control	blue	1x; 400 µL	96	Artificial nucleic acid target	Mix of RNA (approx. 10^7 copies/µl) and plasmid DNA (approx. 1 pg/µl);
Positive Control	red	1x; 50 µL	4	DNA of target analyte	Plasmid DNA of target gene regions (approx. 1 pg/µl)

6. Configurations

The MRSA *direct* PCR is available in the following variants:

Table 3: Configurations of MRSA direct PCR

REFERENCE NUMBER	CONFIGURATION	TARGETS	IC CHANNEL
FBC131-Ax	suitable for use on a qPCR instrument with at least 5 detection channels (**Cyan500**/FAM/HEX/Red610/Cy5)	MRSA; VRSA; MSSA	Cyan500
FBC131-Bx	suitable for use on a qPCR instrument with at least 5 detection channels (FAM/HEX/Red610/Cy5/**Cy5.5**)	MRSA; VRSA; MSSA	Cy5.5
FBC131-Cx	suitable for use on a qPCR instrument with at least 4 detection channels (FAM/HEX/Red610/Cy5)	MRSA; MSSA	HEX

7. Additional Equipment and Reagents (not provided)

- qPCR cycler (with at least 4 detection channels)
- Disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- Sterile filter-tips for PCR testing (DNA/RNA- free)
- Table centrifuge
- DNA isolation kit (IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 by PerkinElmer chemagen Technologie GmbH or similar device); not mandatory (see chapters 11/ 12 test procedure)
- Negative Control (no-template control, molecular grade water, or any other negative control according to the laboratory´s standard procedure)
- Molecular grade H_2O for swab solubilisation

8. Transport, Storage and Stability

The MRSA *direct* PCR test is shipped on dry ice. Solution B must be kept at +2 °C to +8 °C in the dark upon receipt. Solution A, Internal Control and Positive Control must be stored at -25 °C to -18 °C in the dark immediately after receipt. All reagents must be kept at +2 °C to +8 °C during procedure steps. Exposure to light should be avoided. You may thaw and refreeze the kit components up to 5 times. Solution B must not be subjected to repeated freeze–thaw cycles. The package bears an expiry date, after which no quality guarantee can be given.

9. Warnings, Safety Precautions and Additional Information Access

The MRSA *direct* PCR test is intended for *in vitro* diagnostic use only. The test should only be performed by personnel trained in molecular diagnostic techniques. If the user makes changes to the product or the application instructions, results may not correlate with the intended purpose.

All serious incidents relating to the kit must be notified to the manufacturer and the national competent authority of the EU Member State where the laboratory and/or patient is located.

- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to invalid results.

- All patient samples must be treated as potentially infectious material.
- Discard sample and assay waste that was in contact with patient material according to your local, regional, or national safety regulations.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging.
- Protect reagents from heat, moisture, and light.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Avoid contamination of the test by microorganisms and nucleases (DNases and RNases).
- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- 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.
- The test kits are intended for single use and must not be reused after performing qPCR reaction.
- If contamination of the qPCR cycler is suspected, cleaning and maintenance must be carried out according to the system´s manual.
- In the event of failure of the controls (see chapter 14/Results), the test result cannot be evaluated, and a repeat run should be considered.
- Safety Data Sheets (SDS) are available for download via https://frizbiochem.de/downloads/
- Pending EUDAMED entry, the Safety and Performance Summary (SSP) can be downloaded via www.frizbiochem.de

10. Specimen Collection, Handling, Transport, and Storage

Proper sample collection, storage and transport are critical for PCR analyses in general. Inadequate sample collection, improper sample handling and/or transport can lead to false negative results. Therefore, ensure that:

- The instructions of the medical laboratory and/or the manufacturer of the sample collection system for sample collection, transport and storage are followed.
- The sample collector is instructed to collect the sample in accordance with applicable national laws, regulatory requirements and the instructions for use of the sampling tool.

11. Test Procedure with DNA extraction

11.1 Important Points before Starting

This test is intended for use with specimens collected using swabs (e.g., nasal, throat, skin, or wound swabs) and with bacterial culture material. The use of other specimen types may result in invalid or unreliable results.

To ensure validity of each qPCR run, one Positive Control and one Negative Control must be included. The Positive Control consists of Plasmid DNA of target gene regions, whereas the Negative Control (e.g. molecular grade water) must be provided by the user. Failure of either the Positive or Negative Control invalidates the qPCR run, and results obtained from such runs must not be reported.

Appropriate nucleic acid extraction methods must be conducted prior to using this assay. DNA extraction reagents are not part of the MRSA direct PCR kit. Performance evaluation studies have been conducted using the IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 on a chemagic™ 360 instrument (PerkinElmer chemagen Technologie GmbH) for DNA isolation.

11.2 Test Procedure with DNA extraction

Solution A, PC and IC are stored at -25 °C to -18 °C in the dark. Solution B is stored at +2 °C to +8 °C in the dark. All reagents must be kept at +2 °C to +8 °C during procedure steps.

Table 4: Test procedure of MRSA direct PCR with DNA extraction

TEST PROCEDURE	
	Sample preparation
1	Thaw Internal Control only.
2	Add Internal Control to the nucleic acid extraction in accordance with the laboratory´s standard procedure (e.g., add 4µL/sample to lysis buffer; add one additional water sample and use as Negative Control)
3	Put the Internal Control back in the kit box in the deep freezer.
4	Perform nucleic acid extraction according to your laboratory's standard procedure.
	qPCR
5	Thaw Solution A and Positive Control only.
6	Pipette 10 µL/well of Solution A into the PCR microtiter plate/reaction tubes. (If IC is not added to sample during extraction, 0.1 µl IC/sample could be added to Solution A).
7	Add 10 µL/well of eluate from nucleic acid extraction; add 10 µL Positive Control per run; add 10 µL Negative Control per run.
8	Put Solution A and the Positive Control back in the kit box in the deep freezer.
9	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
10	Briefly centrifuge the microtiter plate or reaction tubes.
11	Place the filled plate/reaction tubes in the qPCR cycler.
12	Start program.

12. Test Procedure without DNA extraction

12.1 Important Points before Starting

This test is intended for use with specimens collected using swabs (e.g., nasal, throat, skin, or wound swabs) and with bacterial culture material. The use of other specimen types may result in invalid or unreliable results.

To ensure validity of each qPCR run, one Positive Control and one Negative Control must be included. The Positive Control consists of Plasmid DNA of target gene regions, whereas the Negative Control (e.g. molecular grade water) must be provided by the user. Failure of either the Positive or Negative Control invalidates the qPCR run, and results obtained from such runs must not be reported.

For direct testing Dry swabs, Amies Agar gel swabs, Amines solution, Phosphate-buffered saline (PBS), NaCl, and Tryptic Soy Broth (TSB) have been validated. Samples provided in Copan UTM-RT System (UTM-RT), Yocon VTM/UTM, IsoFlow Steath Fluid (Beckman Coulter) were successfully tested with other Lab *direc*t qPCR assays.

The use of other media may lead to false-negative or invalid results. Samples containing chaotropic salts, such as guanidinium thiocyanate or polihexanide at a concentration of 0.25 µg/mL, must not be used (see chapter 15/Limitations of the method).

12.2 Test Procedure without DNA extraction

Solution B is stored at +2 °C to +8 °C in the dark. Solution A and PC are stored at -25 °C to -18 °C in the dark. All reagents must be kept at +2 °C to +8 °C during procedure steps.

Table 5: Test procedure I of MRSA direct PCR without DNA extraction

TEST PROCEDURE I	
	Sample preparation for dry swabs or Agar gel swabs
1	Solubilise swab in 250 µl molecular grade H_2O.
	qPCR
2	Thaw Solution A, and Positive Control only.
3	Take Solution B out of the fridge.
4	Prepare the reaction mix in a separate vial by combining the following reagents per sample: o 10 µL Solution A o 0.2 µL Solution B o 0.1 µL Internal Control Include two additional reactions for the Positive Control and Negative Control. Mix gently and briefly. Centrifuge if needed. Do not vortex.
5	Pipette 10 µL/reaction of the reaction solution into each PCR reaction tube/well of the microtiter plate.
6	Add 10 µL of solubilised sample per reaction; add 10 µL Positive Control per run; add 10 µL Negative Control per run.
7	Put Solution A and the Positive Control back in the kit box in the deep freezer.
8	Put Solution B back in the fridge.
9	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
10	Briefly centrifuge the microtiter plate or reaction tubes.
11	Place the filled plate/reaction tubes in the qPCR cycler.
12	Start program.

Table 6: Test procedure II of MRSA direct PCR without DNA extraction

TEST PROCEDURE II	
	Sample preparation for swabs provided in transport solutions
1	Use transport solution as provided.
	qPCR*
2	Thaw Solution A, and Positive Control only.
3	Take Solution B out of the fridge.
4	Prepare the reaction mix in a separate vial by combining the following reagents per sample: o 10 µL Solution A o 0.2 µL Solution B o 0.1 µL Internal Control o 5 µL H_2O Include two additional reactions for the Positive Control and Negative Control. Mix gently and briefly. Centrifuge if needed. Do not vortex.
5	Pipette 15 µL/reaction of the reaction solution into each PCR reaction tube/well of the microtiter plate.
6	Add 5 µL sample per reaction; add 5 µL Positive Control per run; add 5 µL Negative Control per run.
7	Put Solution A and the Positive Control back in the kit box in the deep freezer.
8	Put Solution B back in the fridge.
9	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
10	Briefly centrifuge the microtiter plate or reaction tubes.
11	Place the filled plate/reaction tubes in the qPCR cycler.
12	Start program.

* If the sample is not provided in Amies solution, Test Procedure I may also be used starting with Step 2.

13.Instrument Settings

The following thermal profile is to be used (see Table 6). Channel settings depend on the qPCR cycler used and should be checked before starting the analysis (see Table 7 and 8).

FBC131-Ax is suitable for qPCR cyclers with at least the five channels (**Cyan 500**, FAM, HEX, Red 610, and Cy5), FBC131-Bx is suitable for qPCR cyclers with at least the five channels (FAM, HEX, Red 610, Cy5, and **Cy5.5**), and FBC131-Cx is suitable for qPCR cyclers with at least the four channels (FAM, HEX, Red 610, and Cy5).

Make sure to activate the detection mode of your qPCR cycler before starting the analysis.

The MRSA *direct* PCR test was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™. In general, Lab *direct* qPCR tests are compatible with other qPCR cyclers (e.g., MIC Cycler, AJ qTOWER, LightCycler® 96 or 480 I).

*Table 7: Instrument Settings of MRSA direct PCR**

STEPS	TEMPERATURE [°C]	TIME	ACQUISITION	#CYCLES
Initial step**	37	2 min	-	1
Initial step (optional)***	55	5 min	-	1
Denaturation	95	3 min	-	1
Amplification	95	5 sec	-	45
	60	30 sec	on	

** If required, the amplification step can be adapted to a two-step cycling protocol by splitting the single 60°C/30sec step into one 60°C/15sec and one 72°C/15sec step.*

*** only for Test Procedure without DNA extraction*
**** only for mixed RNA-/DNA runs*

Table 8: Channel settings of MRSA direct PCR (Ref. No.: FBC131-Ax and FBC131-Bx)

					FBC131-Ax	FBC131-Bx
	MRSA	**VRSA**	**MRSA**	***S. aureus* (MSSA)**	**Internal Control (IC); Amplification Control**	
	orfX	vanA	mecA/mecC	spa	artificial NA	
Reporter Dye	FAM	HEX	Red 610	Cy5	CY500	Cy5.5
Colour	green	yellow-green	orange	red	cyan	far-red
Emission [nm]	520	560	610	670	480	700
Quencher	Black Hole Quencher					

Table 9: Channel settings of MRSA direct PCR (Ref. No.: FBC131-Cx)

				FBC131-Cx
	MRSA	**MRSA**	***S. aureus* (MSSA)**	**Internal Control (IC); Amplification Control**
	orfX	mecA/mecC	spa	artificial NA
Reporter Dye	FAM	Red 610	Cy5	HEX
Colour	green	orange	red	yellow-green
Emission [nm]	520	610	700	560
Quencher	Black Hole Quencher			

14. Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value (see Figure 1). A positive signal is characterized by a sigmoidal curve exhibiting both a baseline phase and an exponential phase. Signals that do not exhibit this curve pattern, despite having a Ct value, may be considered negative. The threshold shall be set within the exponential phase. It should be noted that in the case of low positive samples, the plateau phase might not be visible due to the cut off after 45 cycles. Thus, the presence of a plateau phase is not an essential requirement for defining a positive signal in such cases.

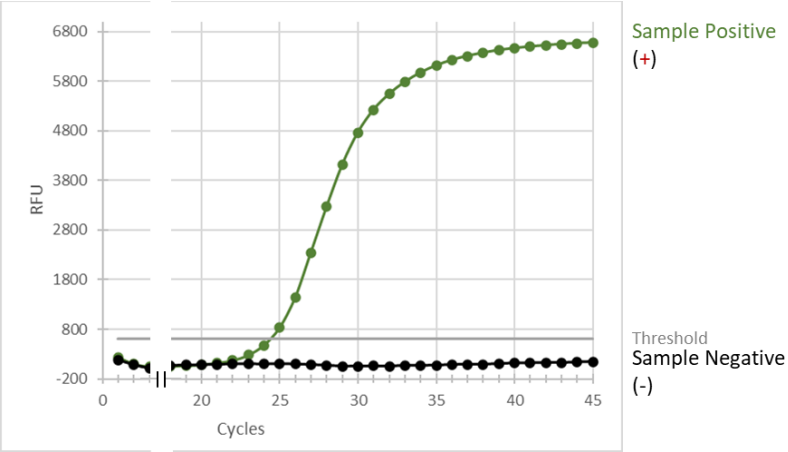


Figure 1: Exemplary amplification curves

- The Negative Control must not show no amplification curves in all channels except for the IC/Amplification Control channel.
- The Positive Control must show amplification curves in all target channels. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems
- Test Procedure with DNA extraction: If the IC is directly included in Solution A, an amplification curve must also be observed in the IC channel of the Positive Control. The one exception is in cases of high pathogen load, where competition for reagents may prevent amplification of the amplification control. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- Test Procedure without DNA extraction: The Amplification Control integrated into Solution B is expected to show positive amplification in both positive and negative samples as well as PC. The one exception is in cases of high pathogen load, where competition for reagents may prevent amplification of the amplification control.

For distribution of results within the channels see Table 9 and Table 10.

Table 10: Results of MRSA direct PCR (Ref. No.: FBC131-Ax and FBC131-Bx)

				FBC131-Ax / FBC131-Bx			
MRSA	**VRSA**	**MRSA**	***S. aureus* (MSSA)**	**Internal Control (IC); Amplification Control**			
orfX	vanA	mecA/mecC	spa	artificial NA			
FAM	HEX	Red 610	Cy5	CY500	Cy5.5	**Result**	**Interpretation**
–	–	–	+	+		Valid	MSSA detected.
+	–	+	+	+		Valid	MRSA detected.
–	+	–	+	+		Valid	VRSA detected.
+/–	+/–	+/–	+/–	+		Valid	orfX and/or vanA and/or mecA/mecC and/or spa detected.[A]
–	–	–	–	+		Valid	No MRSA, VRSA or MSSA detected.
+/–	+/–	+/–	+/–	–		Invalid	The test result can not be evaluated.

[A] *The presence of* orfX and/or vanA and/or *mecA/mecC and/or spa may originate from non-target organisms.*

Table 11: Results of MRSA direct PCR (Ref. No.: FBC131-Cx)

FBC131-Cx

MRSA	MRSA	*S. aureus* (MSSA)	Internal Control (IC); Amplification Control		
orfX	mecA/mecC	spa	artificial NA		
FAM	Red 610	Cy5	HEX	**Result**	**Interpretation**
–	–	+	+	Valid	MSSA detected.
+	+	+	+	Valid	MRSA detected.
+/–	+/–	+/–	+	Valid	orfX and/or mecA/mecC and/or spa detected.[A]
–	–	–	+	Valid	No MRSA or MSSA detected.
+/–	+/–	+/–	–	Invalid	The test result can not be evaluated.

[A] *The presence of* orfX and/or vanA and/or *mecA/mecC and/or spa may originate from non-target organisms.*

15. Limitations of the Method

The results support the differential diagnosis of infection with MRSA and VRSA as well as MSSA. The bacterial DNA may be detectable in clinical specimens from symptomatic and asymptomatic individuals and can be present during both acute and non-acute stages. Detection of bacterial DNA does not differentiate between active infection and colonization; therefore, test results should be interpreted in conjunction with clinical findings, patient history, and other laboratory results.

Positive results indicate the presence of the pathogen, but do not exclude a co-infection with other pathogens.

A negative result does not exclude the presence of the pathogen(s) as results depend on correct sampling, the absence of inhibitors and sufficient nucleic acid to be detected. Invalid results may be obtained if the sample contains inhibitors that prevent lysis, extraction, transcription and/or amplification or detection of the target nucleic acids.

Test results should always be seen in the context of the clinical findings and should not be used as the only basis for diagnosis, treatment, or other patient management decisions. Therapeutic consequences of the diagnostic results must be drawn in relation to the clinical findings by an appropriate healthcare professional.

The detection of analyte target does not mean that they are the causative agents of clinical symptoms.

Mutations or polymorphisms in primer and probe binding regions can interfere with the detection of new variants that may result in false negative results.

The detection of a target analyte does not exclude the possibility of a co-infection with another target analyte since competitive interference may occur for VR*SA* at low concentration (3x LoD) when MRSA concentration is ≥10^6 DNA cp/ml. As a result of these findings, it is recommended that the detection of MRSA should not exclude the possibility of a co-infection with VRSA.

Interference testing demonstrated that the presence of blood clots/blood at a concentration of 5% (v/v) per reaction may affect the detection of the orfX target, with positive signals observed in only 2 of 3 replicates. All other targets were detected in 3 of 3 replicates under the same conditions.

Furthermore, mucin at a concentration of 0.01 mg/µL per reaction inhibited detection of the orfX target, whereas all other targets were detected in 3 of 3 replicates. Therefore, specimens containing elevated levels of blood and/or mucin may lead to reduced sensitivity for orfX detection and should be interpreted with caution.

Furthermore, polihexanide at a concentration of 0.25 µg/mL per reaction produced false-positive signals in the absence of target DNA. Therefore, specimens potentially contaminated with polihexanide-containing antiseptic solutions may yield invalid results and should be interpreted with caution. As a best practice, contamination with polihexanide-containing antiseptic solutions should be avoided.

16. Analytical Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of freshly cultivated and diluted fluid cultures. The analytes were tested in 20 respectively 14 (for concentrations higher 5 CFU/rxn) replicates per concentration on a Bio-Rad CFX Opus 96™. The 95% confidence interval (95 CI) was determined using Probit Regression with the GraphPad Prism 10.0.2 software (see Table 10). A total of 20 MRSA strains representing frequently occurring SCCmec types and subtypes were included in the analytical performance evaluation. Strain 24-02495 carried the mecC gene, whereas all other strains carried the mecA gene.

Table 12: Limit of Detection of MRSA direct PCR

		orfX			*mecA/mecC*			*spa*			*vanA*		
SCCmec type	*S. aureus* strain	LoD (CFU/rxn)	Upper Limit	Lower Limit	LoD (CFU/rxn)	Upper Limit	Lower Limit	LoD (CFU/rxn)	Upper Limit	Lower Limit	LoD (CFU/rxn)	Upper Limit	Lower Limit
IV (multiple subtypes)	MRSA 24-02594	12.0	31.7	3.6	10.9	29.3	3.2	12.5	30.8	4.0		n.a.	
VI	MRSA 24-01421	2.5	2.7	2.2	2.9	4.8	1.5	3.9	5.8	2.5		n.a.	
IIa	MRSA 24-01360	4.1	5.2	2.9	3.6	5.8	2.0	6.3	15.0	2.5		n.a.	
IVa	MRSA 24-01936	2.1	11.1	0.0	2.1	11.1	0.0	2.2	5.2	1.1		n.a.	
IVj	MRSA 24-00851	12.6	40.9	3.4	11.1	23.8	4.5	7.7	10.6	5.3		n.a.	
VIII (multiple subtypes	MRSA 24-01673	2.8	5.0	1.4	1.5	1.9	1.2	3.6	4.8	2.7		n.a.	
XIII	MRSA 24-00586	12.2	49.3	1.4	6.8	14.6	2.6	5.9	11.2	2.5		n.a.	
IV	MRSA 24-01892	11.6	43.4	2.5	8.1	16.7	3.3	8.4	20.1	2.8		n.a.	
IIa	MRSA 24-02445	9.8	24.7	3.5	11.6	69.2	2.0	10.2	21.0	4.5		n.a.	
IVa	MRSA 24-02282	3.6	9.6	1.7	4.2	17.3	1.2	3.4	6.8	1.5		n.a.	
IVj	MRSA 24-00300	3.9	7.8	1.7	3.9	8.9	1.6	4.4	8.1	2.3		n.a.	
XIII	MRSA 24-02495	11.7	44.8	2.7	12.1	46.3	2.7	7.9	26.4	2.1		n.a.	
III	MRSA 24-01611	10.2	35.8	2.7	6.1	10.3	3.5	18.1	88.5	3.5		n.a.	
Vc	MRSA 24-02575	6.1	16.0	2.1	5.2	8.5	3.0	7.8	29.8	1.9		n.a.	
V	MRSA 24-02872	4.6	12.9	1.6	11.0	94.3	1.5	5.6	11.0	2.6		n.a.	
IV (multiple subtypes)	MRSA 24-02280	11.5	47.2	2.7	11.0	72.3	1.8	9.8	28.1	3.0		n.a.	
Ia	MRSA 07-03162	3.3	5.8	1.8	2.1	2.9	1.4	4.7	7.8	2.6		n.a.	
IV (multiple subtypes)	MRSA 24-02810	7.8	25.6	2.1	5.2	9.3	2.6	6.0	13.5	2.2		n.a.	
n.a.	VRSA NR-46412	14.8	38.9	4.6	14.4	38.4	4.3	17.5	50.9	4.8	11.0	23.0	3.8
n.a.	VRSA NR-46422	13.4	26.3	6.0	10.0	10.0	4.4	17.0	43.8	5.4	2.1	3.6	1.1

16.2 Other Analytical Performance Parameters

Other analytical performance data, such as analytical specificity including cross-reactivity, endogenous and exogenous interfering substances, inclusivity and competitive interference as well as precision (repeatability and reproducibility) were performed and demonstrated no impact on assay performance with all test acceptance criteria met.

17. Diagnostic Performance

A total of 97 samples were included in the diagnostic performance evaluation. Samples intended for MRSA testing (66) as well as samples for MSSA testing (20) testing were analyzed directly, without any additional pre-treatment or nucleic acid extraction. Samples intended for VRSA (10) were tested with nucleic acid extraction prior to analysis, in accordance with the Instructions for Use of the comparator assay. Extraction was performed using the chemagic™ Viral DNA/RNA 300 Kit H96 (IVD-1033-S, Revvity) on the chemagic™ 360 instrument, following the manufacturer's instructions.

Direct samples or extracted nucleic acid eluates were analyzed in parallel using the MRSA *direc*t PCR assay and a CE-marked comparator assay from another manufacturer on a Bio-Rad CFX Opus 96™ instrument. In cases of discrepant results between the two assays, discrepancy resolution was performed using an additional CE-marked assay from a different manufacturer or sequencing, as appropriate.

Diagnostic sensitivity and specificity are summarized in the following:

MRSA samples

		positive	negative	
MRSA *direct* PCR	positive	37	0	Sensitivity: 100.0 % (90.5 % - 100.0 %)
	negative	0	29	Specificity: 100.00 % (88.1 % – 100.00 %)

VRSA samples

		positive	negative	
MRSA *direct* PCR	positive	6	0	Sensitivity: 100.0% (54.0 % - 100.0 %)
	negative	0	4	Specificity: 100.0 % (39.8 % – 100.0 %)

MSSA samples

		positive	negative	
MRSA *direct* PCR	positive	18	0	Sensitivity: 100.0% (79.4 % - 100.0 %)
	negative	0	2	Specificity: 100.0% (15.8 % – 100.0 %)

The diagnostic sensitivity of the MRSA *direct* RT-PCR test using DNA extraction is dependent on the DNA extraction method used to isolate DNA from biological specimens.

CE 0123 This product complies with the requirements of the Regulation (EU) *2017*/746 (*IVDR*) for in vitro diagnostic medical devices.

18. Symbols

Please note following symbol descriptions according to EN ISO 15223-1.

Graphic	Title	Description
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Manufacturer	Indicate the medical device manufacturer.
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.
	Use-by date	Indicates the date after which the medical device is not to be used.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.
	CE marking European Conformity	--
	Catalogue number (Reference number)	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Content sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.
	Do not reuse	Indicates a medical device that is intended for one single use only.
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Keep away from sunlight	Indicates a medical device that needs protection from light sources.
	Unique device identifier	Indicates a carrier that contains unique device identifier information.

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19. Technical Assistance and Contact Information

For technical assistance, product support, or questions related to the performance or interpretation of this test, users may contact the manufacturer directly.

Manufacturer:	FRIZ Biochem GmbH
Address:	Floriansbogen 2-4 / 82061 Neuried / Germany
Telephone:	+49 (0) 89 72 44 09 0
Email (Technical Assistance):	support@frizbiochem.de
Email (General Enquiry):	info@frizbiochem.de
Website:	www.frizbiochem.de

Technical support is available during regular business hours, and all inquiries will be handled by qualified personnel. Users are encouraged to contact the manufacturer immediately in the event of suspected malfunction, unexpected results, or any issue requiring expert clarification.

20.References

[1] B. Cookson, “Five decades of MRSA: controversy and uncertainty continues,” The Lancet, vol. 378, 2011.

[2] H. M. Biggs et al., “Trends in incidence and epidemiology of MRSA bacteremia, 2005–2022,” Open Forum Infectious Diseases, vol. 12, 2025.

[3] F. D. Lowy, “Antimicrobial resistance: the example of Staphylococcus aureus,” Journal of Clinical Investigation, vol. 111, 2003. Available:

[4] RKI, Epidemiologisches Bulletin 40, 7. Oktober 2021

[5] https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Staphylokokken_MRSA.html, 16 May 2022

[6] García-Álvarez, L et al. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011, 11: 595–603.

[7] Luo, J. et al. Accurate Detection of Methicillin-Resistant Staphylococcus aureus in Mixtures by Use of Single-Bacterium Duplex Droplet Digital PCR. J Clin Microbiol. 2017 Oct; 55(10): 2946–2955.

[8] CDC’s 2019 Antibiotic Resistance Threats Report, Fact sheet: Methicillin-resistant Staphylococcus aureus

[9] Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2009 Sep;7(9):629-41. doi: 10.1038/nrmicro2200. PMID: 19680247; PMCID: PMC2871281.

[10] Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998 Aug 20;339(8):520-32. doi: 10.1056/NEJM199808203390806. PMID: 9709046.

[11] Centers for Disease Control and Prevention, “Laboratory Testing for Methicillin-resistant Staphylococcus aureus (MRSA),” 2025.

[12] Y. Uehara, “Current status of staphylococcal cassette chromosome mec (SCCmec),” Antibiotics, vol. 11, 2022.

[13] M. Wu et al., “Prevalence of methicillin-resistant Staphylococcus aureus…,” PLoS ONE, 2019.



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