Instructions for Use

In-vitro-Diagnostic IVD

The MRSA *direct* PCR is a real-time PCR test for in vitro detection of DNA from methicillin-resistant *Staphylococcus aureus* (MRSA).

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

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1. Intended Use

The MRSA *direct* PCR test is an in vitro real-time polymerase chain reaction (PCR) assay for the qualitative detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal swabs. The test is intended to support the diagnosis as well as the prevention and control of MRSA infections in healthcare settings. Negative results do not rule out MRSA infection and should not be used as the sole basis for diagnosis. The test is intended for use in qualified laboratories by personnel trained in molecular diagnostic techniques.

2. Pathogen Information

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.[1]

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.[2] The SCCmec cassette type XI (SCCmec XI) contains another mecA homolog, also termed mecC or mecLGA251, and was first described in 2011.[3] 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.[4]

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.[5] 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.

3. Testing Principle

The MRSA *direct* PCR test is designed to detect MRSA directly from the swab on a commercially available qPCR cycler. It consists of a test kit with solutions that contain all necessary chemicals (buffer components, substances for PCR including an internal process control). After adding untreated patient sample to the reaction solution, the PCR analysis is carried out directly in the qPCR device.

The MRSA *direct* PCR test contains probes specific for the following targets: (1) MREJ, (2) mecA, (3) mecC, and (4) 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 the intact probes. The analysis is performed by determining Ct (cycle threshold) values. The Ct value describes the cycle in which the signal rises above a certain threshold for the first time. The more target copies (here: bacterial DNA) are present in the sample, the lower the Ct value. The Internal Control (IC) allows the detection of PCR inhibition. The buffer contains additives for bacterial lysis and enhancement of PCR efficiency. Patient samples can be analysed directly without prior DNA extraction.

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

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

[3] 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.

[4] 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.

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

4. Package Content

The reagents of each vial set are sufficient for 96 reactions. Each set contains:

Material	Lid Colour	Comment
Solution A	blue	Reaction buffer, lysis enzymes, primer and probes
Solution B	yellow	Internal Control (artificial DNA target), polymerase
Negative Control	colourless	RNA/DNA-free molecular grade water
Positive Control	green	Target-specific DNA

Additionally, a quick start protocol is provided per packing unit.

5. Additional Equipment and Reagents

- qPCR cycler
- disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- Pipette tips with filter (DNase/RNase-free)
- Table centrifuge

6. Transport, Storage and Stability

The MRSA *direct* PCR test is shipped on dry ice. All components must be stored at -25 °C to -18 °C in the dark immediately after receipt. Reagents should be handled at +2 °C to +8 °C and used within 4 hours. Repeated thawing and freezing of the components – more than once – shall be avoided. If necessary, aliquoting of the test components after the first thawing is recommended. The package bears an expiry date, after which no quality guarantee can be given.

7. Warnings and Safety Precautions

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. Cross-contamination can lead to false test results. Add patient samples and controls carefully. Ensure that reaction preparations are not carried over from one well to the next. If the user makes substantial changes to the product or the application instructions, results may not correlate with the intended use.

- ➢ Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to incorrect 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.
- ➢ The concentration specifications and incubation times of the manufacturers must be followed.
- ➢ 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.
- ➢ Good laboratory practice should be followed during the test.
- ➢ 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.
- ➢ If contamination of the qPCR device is suspected, cleaning and maintenance must be carried out according to the qPCR system manual.
- ➢ Safety Data Sheets (SDS) are available on request from FRIZ Biochem.

8. Important Points before Starting/Sample Material

Follow the procedures in these instructions for use: reliable results can only be guaranteed if proper procedures are used for sampling (nasal swabs according to manufacturer's instructions), transportation, storage, and handling of the samples.

This test is intended for the detection of MRSA in nasal swabs. Other specimen types may cause false negative or invalid results.

The patient sample needs to be solubilised to be directly used for PCR (without prior DNA isolation). For direct testing, the following solubilisations or transport media have been validated as suitable:

- 0.9% NaCl solution (with or without 5 mM TCEP),
- 0.45% NaCl solution,
- molecular grade water,
- Amies solution,
- UTM (Copan),
- Yocon UTM,
- BD™ Universal Viral Transport System,
- IsoFlow Sheath Fluid (Beckmann Coulter), and
- PBS buffer

Other solubilisation media may lead to false negative or invalid results. Samples in solutions with chaotropic salts (such as guanidinium thiocyanate) must not be used.

The presence of various interfering substances can lead to false negative or invalid results. The MRSA *direct* PCR test includes an Internal Control (artificial DNA target) to detect samples containing substances that interfere with PCR amplification.

In every PCR run one Positive Control and one Negative Control should be included. The Positive Control FBC111-PC consists of target-specific DNA. A failed Positive or Negative Control will invalidate the PCR run and the results must not be reported.

9. Procedure

Thaw all reagents directly before starting the test, keep them cool (+2 °C to +8 °C), and use within 4 hours.

	Procedure for direct testing (without DNA extraction)
1	*Optional: mix sample 1:1 with molecular grade water*
2	*Optional: heat sample at 95 °C for 5 minutes*
3	Prepare the reaction solution: add 100 µL of **Solution B** to the vial of **Solution A**; mix/shake briefly and centrifuge if necessary. Do not vortex!
4	Pipette 15 µL of the **reaction solution** into each PCR reaction tube/well of the microtiter plate.
5	Add 10 µL of **Positive Control** and 10 µL of **Negative Control** per run in respective wells. Add 10 µL of **solubilised patient sample** per remaining reaction tube/well of the microtiter plate.
6	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
7	Briefly centrifuge the microtiter plate or reaction tubes.
8	Place the filled plate/reaction tubes in the qPCR cycler.

10. Instrument Settings

The MRSA *direct* PCR test was validated with the Roche LightCycler® 480 II and with the BioRad qPCR cycler CFX Opus 96™. In general, FRIZ *direct* PCR tests are compatible with any qPCR cycler (e.g., MIC Magnetic Induction Cycler, ABI QuantStudio 5, AJ qTOWER, Roche LightCycler® 96, Roche LightCycler® 480 I) but these and other cyclers may lead to altered Ct values and results. For basic information on programming your qPCR cycler, please refer to the user manual of the qPCR cycler used. The following thermal profile is to be used.

Steps	Temperature [°C]	Time	Number of cycles
Cell lysis	37	2 min	1x
Initial denaturation	95	5 min	1x
Denaturation	95	10 sec	45x
Amplification/Elongation	60	30 sec	

Channel settings are given below.

	MREJ	mecA	mecC	Internal Control (IC)
Reporter dye	FAM	Cy5	Red 610	HEX
Colour	green	red	orange	yellow-green
Emission	520 nm	670 nm	610 nm	560 nm
Quencher	Black Hole Quencher			

11. Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value.

- The Negative Control must not show amplification curves in all channels except for the IC channel.
- The Positive Control must show amplification curves in all channels. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- The IC for negative samples must show a positive amplification curve with a Ct value comparable to the IC Ct value in the Negative Control.
- The IC for positive samples should show a positive amplification curve with a Ct value comparable to the IC Ct value in the Negative Control.
- A significantly higher IC Ct value in samples compared to the IC Ct value in the Negative Control indicates procedural problems (e.g., purification problems or PCR inhibition).
- A high concentration of detectable DNA in the sample can lead to reduced or absent IC signals. In such cases the result for the IC can be neglected.

MREJ (FAM)	mecA (Cy5)	mecC (Red 610)	IC (HEX)	Result	Interpretation
Positive	**Positive**	Negative	Positive/ Negative	**Positive**	MRSA detected, positive for mecA
Positive	Negative	**Positive**	Positive/ Negative	**Positive**	MRSA detected, positive for mecC
Positive	Negative	Negative	Positive/ Negative	**Negative**	MRSA not detected
Negative	**Positive**	Negative	Positive/ Negative	**Negative**	MRSA not detected
Negative	Negative	**Positive**	Positive/ Negative	**Negative**	MRSA not detected
Negative	Negative	Negative	**Positive**	**Negative**	MRSA not detected
Negative	Negative	Negative	Negative	**Invalid!**	The test result cannot be evaluated.

12. Limitations of the Method

Test results should always be seen in the context of the clinical findings. Therapeutic consequences and hygiene measures of the diagnostic results must be drawn in relation to the clinical findings.

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

A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates the existence of the corresponding target genes.

A negative result does not exclude the presence of MRSA, as results depend on correct sampling, the absence of inhibitors and sufficient DNA to be detected.

Invalid results may be obtained if the sample contains inhibitors that prevent lysis, amplification or detection of target nucleic acids.

There is a risk of false positive results due to cross-contamination by target organisms, their nucleic acids or their amplified product.

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

If only the resistance gene mecA or mecC is detected there may be an infection with CoNS (coagulase-negative staphylococci) since it can also possess the resistance gene mecA/mecC.

In rare cases methicillin-sensitive *S. aureus* mecA/mecC drop-out mutants result in a positive signal for MREJ but a negative signal for mecA/mecC.

In case of a mixed infection of MSSA (methicillin-sensitive *S. aureus*) and CoNS the MRSA *direct* PCR test may show positive results.

The MRSA *direct* PCR test can detect the 12 most abundant MREJ types. The test may not detect other MREJ types and may show negative results.

13. Analytical Performance

13.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of quantified MRSA culture isolates spiked in a negative human nasal matrix. The analytes were tested in 24 replicates per concentration on a Bio-Rad CFX Opus 96. The 95% detection limit (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 9.3.1 software.

MRSA strain	MREJ	SCCmec	mecA/ mecC	LoD (95 CI) (CFU/reaction)
CM05	Type i	n.d.	mecA	5.47 (3.16 – 8.91)
M2884	Type ii	IVg	mecA	9.02 (9.38 – 8.71)
M6040	Type iii	Vx	mecA	12.6 (0.1 – 100.0)
M4500	Type iv	n.d.	mecA	36.4 (0.1 – 100.0)
AR43/3330.1	Type v	IVE	mecA	16.6 (4.5 – 63.7)
M08/0126	Type vi	IVh	mecA	16.9 (5.0 – 52.7)
1309	Type vii	IVa	mecA	5.90 (4.98 – 7.05)
M620	Type ix	IVa	mecA	7.05 (5.08 – 9.95)
M2970	Type LGA251	XI	mecC	37.4 (17.5 – 79.4)
M2885	Type M	IX	mecA	7.26 (5.09 – 11.64)

13.2 Analytical Specificity

The specificity of the test was determined *in vitro* by examining relevant bacterial culture isolates, including methicillin-sensitive coagulase-negative staphylococci (MSCoNS) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) spiked in a negative human nasal matrix. In addition, nucleic acids of relevant non-

staphylococci bacteria, yeasts and viruses were tested. The measurement was performed in triplicates. No cross-reactivities with the following species were detected (all Internal Controls valid):

Methicillin-sensitive coagulase-positive staphylococci (MSSA)		
Staphylococcus aureus VCU006	*Staphylococcus aureus* No. 66 (NRS199)	*Staphylococcus schweitzeri* FSA084
Staphylococcus aureus, Strain MN8		
Methicillin-resistant coagulase-negative staphylococci		
Staphylococcus epidermidis RP62a	*Staphylococcus epidermidis* W23144	*Staphylococcus epidermidis* M23864:W2
Staphylococcus epidermidis NIH05001	*Staphylococcus epidermidis* M0881	*Staphylococcus haemolyticus* DNF00585
Staphylococcus epidermidis VCU013	*Staphylococcus epidermidis* BCM0060	*Staphylococcus epidermidis* VCU014 AKA NRS 849
Staphylococcus epidermidis MR	*Staphylococcus haemolyticus* Shae 2 (NRS69)	
Methicillin-resistant coagulase-positive staphylococci		
Staphylococcus pseudintermedius S32	*Staphylococcus pseudintermedius* KM1381	
Methicillin-sensitive coagulase-negative staphylococci		
Staphylococcus warneri AMC 263	*Staphylococcus epidermidis* VCU036	*Staphylococcus haemolyticus* AKA NRS115
Staphylococcus warneri	*Staphylococcus caprae* C87	*Staphylococcus epidermidis* MR
Staphylococcus equorum	*Staphylococcus petrasii*	*Staphylococcus hominis*
Staphylococcus cohnii	*Staphylococcus rostri*	*Staphylococcus capitis*
Staphylococcus pseudintermedius 9505/46	*Staphylococcus epidermidis* NIHLM020	*Staphylococcus caprae*
Staphylococcus capitis SK14	*Staphylococcus haemolyticus*	*Staphylococcus lugdunensis*
Staphylococcus hominis, Strain SK119	*Staphylococcus saprophyticus* B.C.H.C	*Staphylococcus pasteuri* BM9357
Staphylococcus succinus	*Staphylococcus saprophyticus*	*Staphylococcus sciuri ssp. Rodentium*
Staphylococcus sciuri	*Staphylococcus condimenti*	*Staphylococcus kloosii* CSCA7
Staphylococcus microti	*Staphylococcus auricularis*	*Staphylococcus vitulinus* CSBO8
Non-staphylococci gram-positive bacteria		
Corynebacterium bovis	*Bacillus cereus*	*Lactobacillus salivarius*
Enterococcus faecium	*Streptococcus mutans*	
Gram-negative bacteria		
Pseudomonas aeruginosa	*Haemophilus influenzae*	*Legionella pneumophila*
Acinetobacter baumannii	*Neisseria meningitidis*	
Yeasts		
Candida albicans		
Viruses		
Influenza A	Parainfluenza 1	Human Coronavirus, NL63
Influenza B	Parainfluenza 2	Enterovirus D68, US/MO/14-18949
SARS-CoV-2	Parainfluenza 3	Rhinovirus
Respiratory Syncytial Virus Type A	Parainfluenza 4 A	Eppstein-Barr-Virus (EBV)
Respiratory Syncytial Virus Type B	Human Coronavirus, 229E	

14. Interfering Substances

To investigate the effect of potentially interfering substances on the performance of the MRSA *direct* PCR testing, the highest concentration of the substance potentially present in nasal swabs was added to a negative clinical matrix with and without MRSA strain M2884 at 3x LoD.

In addition, a control sample with matrix and MRSA strain M2884, but without potentially interfering substance was used as reference. Each condition was tested in triplicates. The following table gives an overview of the potentially interfering substances tested with the MRSA *direct* PCR test, their active ingredient, the amount of medication applied as well as the MRSA *direct* PCR test results. MRSA strain M2884 was clearly identified in the presence of the tested potentially interfering substances at indicated concentrations.

Medication (trade name)	Active ingredient per unit	% (v/v) *	Result SARS-CoV-2	Result Influenza A	Result Influenza B	Result RSV	Result IC
ratioAllerg® Hay fever spray	50 µg Beclomethasone dipopionate	20	3/3	3/3	3/3	3/3	3/3
Otri-Allergie Hay fever spray	50 µg Fluticasone propionate	20	3/3	3/3	3/3	3/3	3/3
MometaHEXAL® Hay fever spray	50 µg Mometasone Fuorate	35	3/3	3/3	3/3	3/3	3/3
NASACORT® Nasal Spray	55 µg triamcinolone acetonide	30	3/3	3/3	3/3	3/3	3/3
Vividrin® akut Nasal Spray	140 µg Azelastine hydrochloride	30	3/3	3/3	3/3	3/3	3/3
Nasivin® Nasal Spray	22,5 µg Oxymetazoline hydrochloride	10	3/3	3/3	3/3	3/3	3/3
NASENSPRAY Heumann	90 µg Xylometazolinhydrochlorid	20	3/3	3/3	3/3	3/3	3/3
Syntaris® Nasal Spray	25 µg Flunisolide	20	3/3	3/3	3/3	3/3	3/3
Rhinocort Topinasal Nasal Spray	64 µg Budesonide	10	3/3	3/3	3/3	3/3	3/3
CromoHEXAL® Eye drops	ca 400 µg Sodium agglomoglicate	5	3/3	3/3	3/3	3/3	3/3
Tobrex® Eye drops	ca 60 µg Tobramycin	5	3/3	3/3	3/3	3/3	3/3
LISTERINE® Cool Mint Mouthwash	Ethanol (n.a.)	25**	3/3	3/3	3/3	3/3	3/3
Chlorhexamed® FLUID 0,1%	15 µg Chlorhexidine bis (D-gluconate)	50	3/3	3/3	3/3	3/3	3/3
Dequonal® Solution	1.5 mg Dequalinium chloride, 3.5 mg Benzalkonium chloride	50	3/3	3/3	3/3	3/3	3/3
Octenident® Mouth rinse solution	Octenidine (n.a.)	25**	3/3	3/3	3/3	3/3	3/3
Human blood	n.a.	5	3/3	3/3	3/3	3/3	3/3
Mucin	n.a.	5	3/3	3/3	3/3	3/3	3/3

* Concentration of medication in the medication-matrix mixture. It is assumed that the volume of one application is completely transferred into the sample.
** Higher concentrations of medication are prone to cause invalid or false negative results

15. Diagnostic Performance

The evaluation of the clinical performance was carried out with serial dilutions (3x, 30x and 300x LoD) of different MRSA isolates possessing different MRE and SCCmec types spiked in a negative human nasal matrix. Negative samples were analysed in parallel with a CE-certified test kit from another manufacturer.

	MRSA positive samples	MRSA negative samples
MRSA *direct* PCR positive	100	0
MRSA *direct* PCR negative	0	50
Sensitivity/Specificity	**Sensitivity 100%**	**Specificity 100%**

16. Note to the User

All serious incidents relating to the device must be notified to the manufacturer and the competent authority of the Member State where the user and/or patient is established.

This product complies with the requirements of the European Directive 98/79/EC for in vitro diagnostics.

17. Symbols

IVD	For use in in vitro diagnostics	REF	Item number
	Manufacturer	Σ	Content sufficient for XY provisions
	Temperature limitation		Biological risk
	Can be used until	LOT	Batch designation
	Follow instructions for use	CONT	Content

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