

# Evaluation of the eazyplex<sup>®</sup>MRSA system for the detection of MRSA and MSSA strains

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## Introduction

The rapid detection and accurate identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains is essential for disease management and outcome as well as infection control and surveillance purposes. The heterogeneity of the SCC<sub>mec</sub> element including the methicillin resistance-encoding gene presents a challenge for the molecular diagnostics of MRSA.

The eazyplex<sup>®</sup> MRSA (AmplexDiagnostics) is a multiple loci targeting, loop-mediated isothermal amplification (LAMP) system for ultra-rapid (within 20 minutes) MRSA detection. It combines species discrimination between *S. aureus* and *S. epidermidis* with the detection of *mecA* and *mecC* genes. To evaluate this amplification system, a well characterized collection of 147 staphylococcal challenge strains was tested.

## Results

**All 49 *mecA*-MRSA, four *mecC*-MRSA and 40 MSSA isolates used in this study were identified correctly as the species *S. aureus*.** A positive cut-off value of 5000 fluorescence-units was reached in all isolates within the first ten minutes.

**The detection or non-detection of the *mecA* and *mecC* genes, respectively, was accurate in all cases.** In case of MRSA the *mecA/mecC* signal was less than 5:30 minutes after the *S. aureus* signal.

Testing of *non-S. aureus* strains demonstrated reliable results. Also the *S. epidermidis* reference strain ATCC 14990 was identified completely correct.

Two type strains (*S. fleurettii* DSM 13212 and *S. hominis* subsp. *novobiosepticus* ATCC 700236) showed unexpected findings of a positive *mecA* signal. However, literature and PCR re-testing (GenoType MRSA, Hain Lifescience) confirmed the presence of the *mecA* gene.

*S. warneri* DSM 20316 showed in two attempts a very late positive *S. aureus* signal, similar to *S. hominis* subsp. *novobiosepticus* ATCC 700236. *S. caprae* DSM 20608 and *S. fleurettii* DSM 13212 showed also a late positive *S. aureus* signal, which could not be confirmed in repetition. The *S. aureus* signals were detected after more than 11 minutes of the test run. In all cases the *S. aureus* signal had no time correlation to the *mecA/mecC* and therefore had no potentially wrong MRSA interpretation.

As a consequence, the eazyplex<sup>®</sup>MRSA was able to reliably detect all staphylococcal strains and isolates.

Tab. 1: Tested MSSA/ MRSA isolates

<i>S. aureus</i> group	Origin	spa-types
MSSA (n=40)	[2]	t002, t005, t008, t015, t021, t024, t056, t084, t091, t097, t122, t156, t159, t160, t166, t177, t213, t216, t223, t230, t252, t331, t360, t362, t605, t1156, t1238, t2364, t3423, t3583, t4230, t4629, t4904, t5160, t9813, t10327, t10353, t10354, t10356, t10388
<i>mecA</i> -MRSA (n=49)	[1]	t003, t032, t008, t002, t034, t011, t045, t014, t022, t8374, t4217, t264, t004, t020, t4881, t001, t1227, t2373, t463, t024, t127, t504, t015, t030, t038, t044, t379, t785, t012, t037, t041, t063, t151, t318, t634, t849, t1107, t114, t1282, t223, t2369, t437, t4417, t481, t535, t578, t651, t6736, t7391
<i>mecC</i> -MRSA (n=4)	[2, 3]	t843, t978, t7189, t1773

Correct identification of species and *mecA/mecC* genes presence in all cases.

Tab. 2: Tested type- and reference strains

<i>S. agnetis</i> DSM 23656 <sup>T</sup>	<i>S. lentus</i> DSM 20352 <sup>T</sup>
<i>S. arlettae</i> DSM 20672 <sup>T</sup>	<i>S. lugdunensis</i> DSM 4804 <sup>T</sup>
<i>S. auricularis</i> DSM 20609 <sup>T</sup>	<i>S. lutrae</i> DSM 10244 <sup>T</sup>
<i>S. capitis</i> subsp. <i>capitis</i> DSM 20326 <sup>T</sup>	<i>S. microti</i> DSM 22147 <sup>T</sup>
<i>S. capitis</i> subsp. <i>capitis</i> DSM 20325 <sup>T</sup>	<i>S. muscae</i> DSM 7068 <sup>T</sup>
<i>S. capitis</i> subsp. <i>urealyticus</i> DSM 6717 <sup>T</sup>	<i>S. nepalensis</i> DSM 15150 <sup>T</sup>
<i>S. caprae</i> DSM 20608 <sup>T</sup>	<i>S. pasteurii</i> DSM 10656 <sup>T</sup>
<i>S. carnosus</i> subsp. <i>carnosus</i> DSM 20501 <sup>T</sup>	<i>S. pettenkoferi</i> DSM 19554 <sup>T</sup>
<i>S. carnosus</i> subsp. <i>utilis</i> DSM 11676 <sup>T</sup>	<i>S. piscifermentans</i> DSM 7373 <sup>T</sup>
<i>S. chromogenes</i> DSM 20454 <sup>T</sup>	<i>S. pseudintermedius</i> LMG 22219 <sup>T</sup>
<i>S. cohnii</i> subsp. <i>cohnii</i> DSM 20260 <sup>T</sup>	<i>S. rostri</i> DSM 21968 <sup>T</sup>
<i>S. cohnii</i> subsp. <i>urealyticus</i> DSM 6718 <sup>T</sup>	<i>S. saccharolyticus</i> DSM 20359 <sup>T</sup>
<i>S. condimentii</i> DSM 11674 <sup>T</sup>	<i>S. saprophyticus</i> subsp. <i>saprophyticus</i> DSM 20229 <sup>T</sup>
<i>S. delphini</i> DSM 20771 <sup>T</sup>	<i>S. saprophyticus</i> subsp. <i>bovis</i> CCM 4410 <sup>T</sup>
<i>S. devriesei</i> CCUG 58238 <sup>T</sup>	<i>S. schleiferi</i> subsp. <i>coagulans</i> DSM 6628 <sup>T</sup>
<i>S. epidermidis</i> ATCC 14990 <sup>T</sup>	<i>S. schleiferi</i> subsp. <i>schleiferi</i> DSM 4807 <sup>T</sup>
<i>S. equorum</i> subsp. <i>equorum</i> DSM 20674 <sup>T</sup>	<i>S. sciuri</i> subsp. <i>carnaticus</i> ATCC 700058 <sup>T</sup>
<i>S. equorum</i> subsp. <i>linens</i> DSM 15097 <sup>T</sup>	<i>S. sciuri</i> subsp. <i>rodentium</i> ATCC 700061 <sup>T</sup>
<i>S. felis</i> DSM 7377 <sup>T</sup>	<i>S. sciuri</i> subsp. <i>sciuri</i> DSM 20345 <sup>T</sup>
<i>S. fleurettii</i> DSM 13212 <sup>T</sup>	<i>S. simiae</i> DSM 17636 <sup>T</sup>
<i>S. gallinarum</i> DSM 20610 <sup>T</sup>	<i>S. simulans</i> DSM 20322 <sup>T</sup>
<i>S. haemolyticus</i> DSM 20263 <sup>T</sup>	<i>S. stepanovicii</i> CCM 7717 <sup>T</sup>
<i>S. hominis</i> subsp. <i>hominis</i> DSM 20328 <sup>T</sup>	<i>S. succinus</i> subsp. <i>casei</i> DSM 15096 <sup>T</sup>
<i>S. hominis</i> subsp. <i>novobiosepticus</i> ATCC 700236 <sup>T</sup>	<i>S. succinus</i> subsp. <i>succinus</i> DSM 14617 <sup>T</sup>
<i>S. hyicus</i> DSM 20459 <sup>T</sup>	<i>S. vitulinus</i> ATCC 51145 <sup>T</sup>
<i>S. intermedius</i> DSM 20373 <sup>T</sup>	<i>S. warneri</i> DSM 20316 <sup>T</sup>
<i>S. kloosii</i> DSM 20676 <sup>T</sup>	<i>S. xylosus</i> DSM 20266 <sup>T</sup>

## Methods

Overall, 53 clinical MRSA strains were tested, which include the most prevalent *spa* types in Germany during a 6-year period [1]. Furthermore, four *mecC*-positive isolates belonging to four different *spa* types t843, t978, t7189 and t1773 [2,3] were covered in this study.

In addition, 40 methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates identified in various German studies [2] and 54 non-*S. aureus* staphylococcal type and reference strains (Tab. 2) were included, comprising different staphylococcal species and subspecies [4].

All 147 staphylococcal challenge strains were tested by the eazyplex<sup>®</sup> MRSA test system of AmplexDiagnostics and were analyzed manually by the integrated Genie Explorer (V. 2.0.5.0) and the test interpretation algorithm (V. 1.2, 01.12.2014). A positive test signal was defined by the increase of fluorescence of more than 5000 units. Test conditions were used as required by the manufacturer. Bacteria used for this test were inoculated directly from blood agar plate.



## Conclusions

- The eazyplex<sup>®</sup>MRSA system is suitable for ultra-rapid identification of MRSA strains and for distinction between *S. aureus* and non-*S. aureus* strains.
- The evaluation of this assay for the direct detection of MRSA from screening samples warrants further investigations.

### Acknowledgement:

We are grateful to Martina Schulte und Anja Hassing for excellent technical assistance.

The test kits were provided free of charge by AmplexDiagnostics GmbH.

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