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# Evaluation of Simplexa™ MRSA Direct (*Focus Diagnostics*) on a large collection of *mec*A- and *mec*C-positive MRSA representing the major MRSA clones circulating throughout the world.

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## Background

Methicillin Resistant *Staphylococcus aureus* (**MRSA**) is one of the major pathogens responsible for **nosocomial** infections, but also for **community-acquired** infections. The **accurate and rapid detection** of nasal carriers is therefore crucial to allow early isolation and/or decolonization of patients in order to **limit infections** as well as **dissemination** of such strains.

For this purpose, **several PCR assays** have been designed to ensure rapid and reliable screening of MRSA in nasal swabs. They classically targeted i) the junction between *orf*X and SCC*mec* cassette harbouring *mec*A, ii) a gene specific to *S. aureus*, and for some assays iii) the *mec*A gene, itself. Unfortunately, misidentifications have been reported in the currently commercialized screening tests due to:

✓ the **diversity of the *orf*X-SCC*mec* junction** related to the huge number of MRSA clones circulating throughout the world as well as the recent emergence of a ***mec*A variant (named *mec*C)**, both causing mismatches with the dedicated primers that may induce false negative results,

✓ the emergence of ***mec*A drop-out** isolates that are former MRSA strains in which residual parts of SCC*mec* cassette are present but from which the *mec*A gene has been excised which induces false positive results with some screening tests,

✓ the possible nasal **co-colonization** with methicillin-susceptible *Staphylococcus aureus* (**MSSA**) and methicillin-resistant coagulase negative *Staphylococcus* (**MRCoNS**), that may induce false positive results.

**The Simplexa™ MRSA Direct assay (Focus Diagnostics) is a commercial kit belonging to the fourth-generation of MRSA screening tests. It is a triplex real-time amplification-based assay that targets: i) a conserved region in a specific *Staphylococcus aureus* (SA) gene *spa*, allowing species identification; ii) both *mec*A and *mec*C genes, allowing methicillin-resistance detection; iii) an exogenous internal control to monitor reaction inhibition and reagent integrity. To avoid misidentification linked to MSSA and MRCoNS co-colonization, results are considered positive for MRSA only when both targets showed positive amplification combined with similar cycle threshold values demonstrating that these targets are present in the same number of copies and so, are harboured by the same MRSA bacteria.**

In this context, using a very diverse collection of MRSA clinical isolates, we **evaluated the ability of Simplexa™ MRSA Direct to accurately detect the *mec*A-positive and *mec*C-positive MRSA isolates**, representatives of the MRSA clones circulating throughout the world.

## Materials and Methods

### Clinical isolates

✓ **4 MSSA used as control**

✓ **148 MRSA collected from 37 countries**

- extensively characterized: *mec*A/*mec*C PCR, *agr* typing, *spa*-typing, SCC*mec* typing DNA microarray (StaphyType, Alere)
- selected to be representative of the major worldwide-circulating MRSA clones covering: 35 clonal complexes (CC), 70 *spa*-types
- including 25 *mec*C-positive strains (SCC*mec* type XI, 4 CC (130, 1943, 425, 49))

✓ Test performed on 2-3 colonies after 24h-growth on blood agar plates

### Simplexa™ MRSA Direct assay

✓ **Real-time PCR**: the **1-hour** assay was performed on a Direct Amplification Disc™ (8 tests) and the 3M Integrated Cycler™


✓ **Targets:**

- exogenous internal control to monitor reaction inhibition and reagent integrity,
- conserved regions of *S. aureus* genome (***spa*** gene),
- methicillin-resistant genes (***mec*A** and ***mec*C**).

✓ The Integrated Cycler Studio Software version 5.0. automatically compares the results (including Ct) of both targets to identify if MRSA or *S. aureus* are present.

## Results - Discussion

**All isolates were identified as *S. aureus*, except one** for which no *spa*-gene amplification was obtained. A **99.34 % agreement** was reported for the Simplexa™ MRSA Direct assay. Complementary sequencing data, recently obtained, highlighted that the unique misidentified isolate harboured a truncated *spa* gene, explaining the absence of amplification.

**All of the 152 clinical isolates**, including representatives of a **very large panel of the MRSA clones (n= 148)** that are **currently circulating throughout the world**, were **accurately classified as methicillin susceptible (n=4) or methicillin resistant (n=148)**.

Among those, **the 25 *mec*C-positive isolates, belonging to the 4 most prevalent clonal complexes (CC130, CC425, CC1943, CC49), were all detected as MRSA.** This result confirms the ability of the primers designed for identifying this new *mec* variant, that is mainly prevalent in animals, but that has been recently reported in severe human infections and that might become of public health importance in the future.

## Conclusion

**The use of the Simplexa™ MRSA Direct assay proved to be very simple and fast directly from isolated colonies. This assay was able to identify all the most prevalent MRSA clones that are circulating throughout the world, including those harbouring the new *mec*C gene. Only one strain was missed due to a deletion in the *spa* gene.**

Combined with data obtained directly on nasal swabs (data not shown here), our study demonstrates the interest of this new molecular test to **screen MRSA harbouring *mec*A or *mec*C**. This assay does not require a highly skilled operator and is designed for users with limited technical skill or experience, typically operators closer to the patient. Using **nasal swab specimens** without nucleic acid extraction, the Simplexa™ Direct assay allows the **detection of patients carrying *Staphylococcus aureus* or MRSA in around an hour**.

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Focus Diagnostics provided all reagents and kits for this study.