Evaluation of the GENSPEED® MRSA Test Kit and its ability to detect the novel *mecA* homologue *mecC*

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GENSPEED® MRSA test kit
The GENSPEED® platform combines a disposable microfluidic test cartridge (test chip) with a compact device for chemiluminescence (CL) based optical detection GENSPEED® reader. Different capture oligo-nucleotides are deposited onto the microfluidic test cartridge (Figure 1).

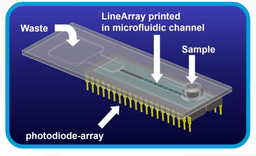

Figure 1. Design of the GENSPEED® microfluid test cartridge

The GENSPEED® MRSA test chip comprises four immobilized target hybridization probes for S. aureus, *S. epidermidis*/*S. haemolyticus*, for the resistance genes *mecA* and *mecC*, and three on-chip controls for performance evaluation. The substrates are bonded onto a proprietary microfluidic channel system and positioned on top of a custom designed photodiode-array. Labeled DNA is hybridized to target molecules, and washing and the final chemiluminescence reaction are controlled via capillary forces in the microfluidic channel system. The temporally and spatially resolved CL-signals from the biochip are detected with a compact readout device (Figure 2).

Figure 2. The GENSPEED ® Reader

Introduction
Early detection of methicillin-resistant Staphylococcus aureus (MRSA) is important in infection control. Traditionally phenotypic cefoxitin resistance has been used as a marker but requires isolation of pure cultures and incubation for 16-20 hours. Automated systems today are only able to detect the classic *mecA* determinant while the newly described homologue *mecC* goes undetected in most systems. The objective of this study was to evaluate the GENSPEED ® MRSA Test Kit using a well characterized MRSA collection including isolates harboring the *mecC* gene. The GenSpeed® MRSA Test Kit is designed to detect both homologues directly from nasal swabs.

Methods
In Denmark all new cases of MRSA are sent to the National Reference Laboratory for Staphylococci for *spa* typing. In this retrospective study all submitted MRSA isolates (N=95) from the Department of Clinical Microbiology (DCM) from Slagelse hospital during May 1, 2010 to April 30, 2011 were included. The DCM serves an area in Denmark where *mecC* prevalence is high (Figure 3).

Figure 3. Location of DCM Slagelse and the catchment area.

The MRSA status was confirmed by multiplex PCR detecting both the *mecA* and *mecC* genes in addition to *pvl* and *spa* (Stegger *et al* 2012). The *spa* gene was sequenced to give the *spa* type, which were annotated to MLST Clonal complex (CC). In addition four *mecC* positive isolates from sheep and cattle and five methicillin-sensitive *S. aureus* (MSSA) were included. The isolates were tested according to the manufacturer's instruction and training, with the exception that pure cultures were used instead of nasal swabs. To obtain the raw signals for each probe a development version of the software was used instead of the standard software. The raw signals were used to determine an appropriate threshold for culture based detection.

Results and discussion
The set represented 27 spa types assigned to CC1, CC22, CC30, CC45, CC5, CC59, CC8, CC80, CC93, ST152/377, CC130, and CC398. Results from the 95 submitted human MRSA cases are shown in Table 1.

Table 1. Number of positive results for the PCR assay and the GENSPEED ® MRSA Test Kit

	GENSPEED results	
PCR results	*mecA*	*mecC*
mecA	89	0
mecC	0	6

The GENSPEED development software correctly identified all *mecA*-MRSA from various genetic lineages and all six *mecC*-MRSA. The latter is of importance since only PCRs and a single commercial system have so far been able to provide fast and reliable detection of the *mecC* type of MRSAs. In addition, all four *mecC* positive animal isolates and the five MSSAs were correctly identified by the MRSA Test Kit.

The evaluation was performed satisfactorily with pure cultures and is expected it to perform equally well directly on nasal swabs.

Reference
Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecA(LGA251). Clin Microbiol Infect. 2012 18(4):395-400.

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