Comparison of the R-Biopharm AG RIDA®GENE MRSA LC2.0 and the Roche LightCycler® MRSA Advanced assay for the laboratory diagnosis of MRSA

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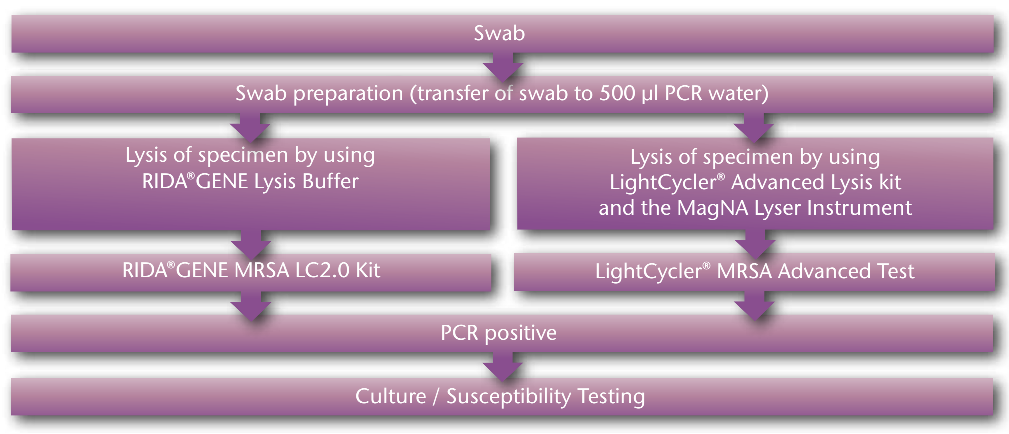
Objectives

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause for infections in hospitals and healthcare settings (HA-MRSA). Infections with MRSA are associated with a higher morbidity, mortality, health care costs and prolonged hospitalization than Methicillin-sensitive Staphylococcus aureus infections. Transmission occurs through health care providers or other patients. Beside HA-MRSA infections also community-acquired MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA) infections emerged. An early, fast and systematic MRSA screening enables a specific treatment of infected patients and introduction of appropriate hygiene interventions prevents MRSA-transmission and spread. Conventional culture-based methods for detection of MRSA require at least 1 day. Real-time PCR assays enable an early and rapid MRSA screening on the day of hospital admission as part of an infection prevention program ("search and destroy" strategy). In a prospective comparison study the performance of the RIDA®GENE MRSA LC2.0 kit (R-Biopharm AG) and the LightCycler® MRSA Advanced Test (Roche) for MRSA screening was evaluated.

Methods

Swabs sent for routine MRSA testing were included in the study. The swabs were transferred to 500 µl PCR water and briefly vortexed. 200 µl of the sample underwent testing on the LightCycler® MRSA Advanced Test (Roche) following the package insert instruction. Another 200 µl of the sample underwent testing on the RIDA®GENE MRSA LC2.0 kit following the package insert instruction. Both assays were run on the LightCycler®2.0.

Figure 1: Study design



Samples with a positive MRSA result by at least one PCR assay were cultured for identification and susceptibility testing using the BD Phoenix™ System. True positive and negative results were defined by an agreement of 2 out of the 3 methods.

The RIDA®GENE MRSA LC2.0 kit simultaneously targets two MRSA DNA regions with fluorogenic target-specific hydrolysis probes, namely the SCCmec:orfX junction as well as the classical mecA and novel mecC gene. In this way the assay is able to identify MRSA, Coagulase negative staphylococci (CoNS) and mecA dropouts. The LightCycler® MRSA Advanced Test targets the integration site of the SCCmec cassette into the S. aureus chromosome (SCCmec:orfX junction) with fluorogenic target specific hybridization probes.

Each PCR method contains an Internal Control to exclude or detect PCR inhibition and to monitor reagent integrity. The Internal Control of the The RIDA®GENE MRSA LC2.0 Kit can also be used as extraction control to monitor inhibitors in the extracted specimen, to assure that adequate amplification has taken place and to confirm that the nucleic acid extraction was sufficient.

Table 1: Analyte target gene and detection channel overview

Target	Detction channel RIDA®GENE MRSA LC2.0 Kit	LightCycler® MRSA Advanced Test
SCCmec:orfX junction	530	610 (T_M Peak 57 - 62 °C)
mecA/mecC	705	None
Internal Control	560	670 (T_M Peak 57 - 62 °C)

Results

Testing of the 227 specimens resulted in an MRSA prevalence of 9.3 % (21/227). Of the 227 specimens, 212 (93.4 %) were concordant.
The LightCycler® MRSA Advanced test yielded 6 false positive results that were negative by both the RIDA®GENE MRSA LC2.0 assay and culture. There was 1 specimen positive by RIDA®GENE MRSA LC2.0 that was negative by LightCycler® MRSA Advanced and culture. 3 specimens were positive by LightCycler® MRSA Advanced and culture but negative by RIDA®GENE MRSA LC2.0. There were 5 specimens that were positive by RIDA®GENE MRSA LC2.0 and culture but negative by LightCycler® MRSA Advanced.

Table 2: Comparison of PCR results

	RIDA®GENE MRSA LC2.0 Kit	LightCycler® MRSA Advanced Test
Total number of samples	227	227
PCR results		
Positive	19 (8.4 %)	22 (9.7 %)
Negative	208 (91.6 %)	205 (90.3 %)
Result definition		
True positive	18 (7.9 %)	16 (7.1 %)
False positive	1 (0.5 %)	6 (2.6 %)
False negative	3 (1.3 %)	5 (2.2 %)
True negative	205 (90.3 %)	200 (88.1 %)

Figure 2: RIDA®GENE MRSA LC2.0 kit compared to a defined reference algorithm

Sens.:	85.7 %
Spec.:	99.5 %
PPV:	94.7 %
NPV:	98.6 %

		Reference algorithm		
		positive	negative	total
RIDA®GENE MRSA LC2.0	positive	18	1	19
	negative	3	205	208
	total	21	206	227

Figure 3: LightCycler® MRSA Advanced Test compared to a defined reference algorithm

Sens.:	76.2 %
Spec.:	97.1 %
PPV:	72.1 %
NPV:	97.6 %

		Reference algorithm		
		positive	negative	total
LightCycler® MRSA Advanced	positive	16	6	22
	negative	5	200	205
	total	21	206	227

Compared to the defined diagnostic truth the sensitivities of the RIDA®GENE MRSA LC2.0 kit and LightCycler® MRSA Advanced Test were 85.7 % and 76.2 %, respectively. Specificities of the RIDA®GENE MRSA LC2.0 kit and the LightCycler® MRSA Advanced Test were 99.5 % and 97.1 %, respectively. The 5 MRSA isolates which were negative by LightCycler® MRSA Advanced Test and positive by the RIDA®GENE MRSA LC2.0 kit and culture were sent to the French National Reference Center for Staphylococci, Lyon for further characterization (StaphyType, Alere Technologies). 4 of the 5 MRSA isolates belonged to the clonal complex 1 and one MRSA belonged to clonal complex 5 and to the pediatric clone.

Table 3: Result of discrepant MRSA isolates characterization

mecA	delta_mecR	ugpQ	ccrA-1	ccrB-1	plsSCC (COL)	Q9XB68-dcs	ccrA-2	ccrB-2	kdpA-SCC	kdpB-SCC	kdpC-SCC	kdpD-SCC	kdpE-SCC	mecI	mecR	xylR	ccrA-3	ccrB-3	merA	merB	ccrAA (MRSAZH47)_probe 1	ccrAA (MRSAZH47)_probe 2	ccrC (85-2082)	ccrA-4	ccrB-4	"MLST clonal compl. Affil."	Strain
POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	CC1	CC1-MRSA-IV, WA MRSA-1/57
POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	CC1	CC1-MRSA-IV, WA MRSA-1/57
POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	CC1	CC1-MRSA-IV, WA MRSA-1/57
POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	CC1	CC1-MRSA-IV, WA MRSA-1/57
POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	CC5	CC5-MRSA-IV, Paediatric clone [sed/j/r+]

Conclusion

- The RIDA®GENE MRSA LC2.0 kit showed higher sensitivity, specificity, PPV and NPV than the LightCycler® MRSA Advanced Test and requires less time to result for MRSA screening.
- The RIDA®GENE MRSA LC2.0 kit can also detect the new mecA homologue (mecC or mecALGA251).
- The RIDA®GENE MRSA LC2.0 kit contains an internal control that can also be used as extraction control to monitor inhibitors in the extracted specimen, to assure that adequate amplification has taken place and to confirm that the nucleic acid extraction was sufficient.

Acknowledgement:
We thank Dr. Frederic Laurent from the French National Reference Center for Staphylococci, Lyon for the characterization of the submitted MRSA isolates.

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