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Evaluation of the R-Biopharm RIDA®GENE PVL real-time PCR assay for the laboratory diagnosis of skin and soft tissue infections caused by PVL

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Objectives

Staphylococcus aureus (SA) is a major cause of nosocomial infections in hospitals and healthcare settings. Since the mid-1990s the number of infections in the population increased with no previous history of medical facility contact. This increase in infections in the population is caused by *Staphylococcus aureus* strains that carry the virulence factor Panton-Valentine leukocidin (PVL). PVL is a cytotoxin encoded by the lukF-PV and lukS-PV genes and contributes to tissue necrosis by lysis of macrophages and neutrophils hence inhibiting the process of wound closure. PVL can be produced by methicillin-sensitive *Staphylocoocus aureus* strains (MSSA) as well as methicillin-resistant *Staphylocoocus aureus* strains (MRSA). MRSA strains that carry the virulence factor PVL are called community-acquired (CA) -MRSA.[1,2] Risk groups for transmission of CA-MRSA or PVL-MSSA are for example families, persons performing close contact sports, persons from educational settings, prisoners and military personnel.[3,4] The clinical manifestation of PVL-positive *Staphylococcus aureus* (PVL-SA) strains are skin and soft tissue infections, particularly recurrent invasive abscesses and in the US, CA-MRSA is already the most common pathogen causing this clinical picture in outpatients.[2] In the diagnosis of skin and soft tissue infections, but also in systemic diseases caused by PVL-SA, it is useful to determine the virulence factor PVL to initiate appropriate therapeutic and hygienic measures. This study evaluated the performance of the RIDA®GENE PVL assay for the detection of skin and soft tissue infections caused by PVL compared to an established in-house real-time PCR assay.

Methods

To evaluate the sensitivity and specificity of the RIDA®GENE PVL real-time PCR assay, 20 known *Staphylococcus aureus*-positive nasal swabs, quantitatively spiked with DNA of PVL-positive strains and 30 known *Staphylococcus aureus*-negative nasal swabs were tested. In a parallel study, 32 known PVL-SA samples (17 MRSA-SA and 15 MSSA-SA samples) and 62 PVL-negative MRSA/MSSA samples were grown on blood agar plates (Figure 1). For both studies, an established in-house real-time PCR assay was defined as gold standard. DNA was analysed on the LightCycler® 480II (Roche). The RIDA®GENE PVL real-time PCR assay is a qualitative assay using hydrolysis probes for the qualitative detection of PVL.

Figure 1 Study design

Table 1 Analyte target gene and detection channel overview

Analyte	Target Gene	Detection Channel Light® Cycler 480II
PVL	lukF-PV	465/510
ICD	Synthetic target DNA sequence	533/580

Results

Using the in-house real-time PCR as gold standard, all 20 known *Staphylococcus aureus*-positive samples, as well as 30 known *Staphylococcus aureus*-negative samples from nasal swabs were correctly identified by the RIDA®GENE PVL real-time PCR assay indicating a sensitivity and specificity of 100 % for the RIDA®GENE PVL real-time PCR assay (Figure 2). Of the 94 samples from culture, 4 samples showed an inhibition in both PCR methods. Compared to the in-house real-time PCR, the RIDA®GENE PVL real-time PCR assay was in 100 % agreement (Figure 3).

Picture 1 RIDA®GENE PVL

Figure 2 In-house RIDA®GENE PVL *vs.* real-time PCR from nose swabs

		In-house real-time PCR +	-	Total
RIDA®GENE PVL	+	20	0	20
	-	0	30	30
	Total	20	30	50

Sensitivity:	100.0 %
Specificity:	100.0 %
PPV:	100.0 %
NPV:	100.0 %

Figure 3 In-house RIDA®GENE PVL *vs.* real-time PCR from culture

		In-house real-time PCR +	-	Total
RIDA®GENE PVL	+	32	0	32
	-	0	62	62
	Total	32	62	94

Sensitivity:	100.0 %
Specificity:	100.0 %
PPV:	100.0 %
NPV:	100.0 %

References:
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2. *Linde HJ and lehn N. Community-associated MRSA:* Klinik, Therapie, Hygiene. Krankenh.hyg. up2date 2008, 3(1):29-44.
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Conclusion

The RIDA®GENE PVL assay is a highly sensitive and rapid qualitative real-time PCR assay for the detection of the PVL gene from human nasal swabs and culture.

The RIDA®GENE PVL real-time PCR shows good correlation with an established real-time PCR for the detection of *Staphylococcus aureus* of skin and soft tissue infections caused by PVL.

Results are available in less than 2 hours.

For research use only! Not for use in diagnostic procedures!

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