RIDA®GENE PVL

real-time PCR

Art. No.: PG0645

100 reactions

For in vitro diagnostic use.

-20 °C

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE PVL is a real-time PCR for the direct, qualitative detection of the PVL-gene (Panton-Valentine leukocidin) from cultures. RIDA®GENE PVL real-time PCR is intended for use as an aid in diagnosis of (skin-/ soft tissue-) infections caused by PVL.

2. Explanation of the test

Staphylococcus aureus is a normal commensal of the skin and mucous membranes of humans that can be detected in 30% of the general population (asymptomatic carriers). Strains of Staphylococcus aureus which have developed antibiotic resistance methicillin and narrow-spectrum ß-lactamase resistant penicillin antibiotics are called MRSA (Methicillin-resistant Staphylococcus aureus). *Staphylococcus aureus* is a major cause of nosocomial infections in hospitals and healthcare settings.[1,2] Since the mid-1990s the number of infections in the population increased with no previously history of medical facilitiy contact. This increase in infections in the population is caused by Staphylococcus aureus strains that carry the virulence factor Panton-Valentine leukocidin. Infections tend to occur in healthy younger people.[3] PVL can be produced by methicillin-sensitive *Staphylocoocus aureus* strains (MSSA) as well as and methicillin-resistant *Staphylocoocus aureus* strains (MRSA). MRSA strains that carry the virulence factor PVL are called CA-MRSA. [4,5] Panton-Valentine leukocidin (PVL) is a bicomponent, poreforming cytotoxin encoded by the lukF-PV and lukS-PV genes. The cytotoxin of PVL lysis macrophages as well as neutrophil granulocytes and contributes to tissue necrosis. The clinical manifestion of PVL-positive *Staphylococcus aureus* strains are skin and soft tissue infections, particularly recurrent invasive abscesses. Rarely necrotizing pneumonia develops with a mortality rate of up to 75%.[3,4,5] Risk groups for transmission CA-MRSA or PVL-MSSA are for example families, persons performing close contact sports, persons from educational settings, prisoners and military personnel.[3,4] In the recent years infections with PVL-MSSA and PVL-MRSA increased worldwide. The HPA Staphylococcus Reference Unit identified in 2010 around 2,200 cases of PVL-SA in England; one half of these are caused by PVL-MSSA, one half of these are caused by PVL-MRSA.[6] The German reference center for staphylococci detected the virulence factor PVL in 1.8% (2005) and 3.1% (2006) of the sent MRSA isolates with suspected CA-MRSA infection. CA-MRSA is now the most common pathogen (>50%) causing skin and soft tissue infections (particularly abscesses) acquired by outpatients in the U.S.[2] In the diagnosis of skin and soft tissue infections, but also in systemic diseases caused by PVL-SA (PVL-MRSA and PVL-MSSA), it is useful to determine the virulence factor PVL to initiate appropriate therapeutic and hygienic

measures. The real-time PCR allows a rapid and early detection of virulence factor PVL.

3. Test principle

The RIDA®GENE PVL assay is a real-time PCR for the direct, qualitative detection of the PVL-gene (Panton-Valentine leukocidin) from cultures. After DNA-isolation, amplification of the gene fragment specific for PVL (lukF-PV, if present) occurs. The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE PVL assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and/or to determine possible PCR-inhibition.

4. Reagents provided

Tab.1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount	Lid Color
1	Reaction Mix	2x 1100 µl	yellow
2	Taq-Polymerase	1x 11 µl	red
D	Internal Control DNA	2x 1800 µl	orange
N	PCR Water	1x 500 µl	white
P	Positive Control	1x 200 µl	blue
L	Lysis Buffer 1	2x 12 ml	colorless

5. Storage instructions

- Protect all reagents, other than the Lysis Buffer 1, from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Lysis Buffer 1 can also be stored at 2 – 8 °C and can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw and fully defrost reagents, other than the Lysis Buffer 1, before using (e.g. in a refrigerator at 2 - 8 °C).
- Lysis Buffer 1 should be fully defrosted and returned to room temperature before use.
- Reagents can sustain up to 5 freeze/thaw cycles without influencing the assay performance. (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all reagents, other than the Lysis Buffer 1, should be stored cold in an appropriate way (2 - 8 °C).

6. Additional equipment and materials required

- Heating Block at 95°C
- Real-time PCR instrument:
  Roche: LightCycler® 2.0, LightCycler® 480II
- Cepheid: SmartCycler®
  Applied Biosystems: ABI 7500
  Abbott: m2000rt
  Stratagene: Mx3000P, Mx3005P
  QIAGEN: Rotor-Gene Q
- RIDA®GENE Color Compensation II (PG0002) for run the LightCycler® 2.0
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves

7. Precautions for users

- For *in vitro* diagnostic use only.
- DNA isolation, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

8. Test procedure

8.1 Sample preparation

8.1.1 DNA Isolation from Cultures

For DNA isolation from culture the following procedure is recommended: Add 200 µl Lysis Buffer 1 into a preparation tube. Collect colonies with an inoculation loop and suspend them in the pre-pipetted Lysis Buffer 1. Cut or break the inoculation loop stem. Cap the preparation tube tightly and vortex strongly for 60 sec. Heat and shake the preparation tube at 95 °C for 10 min in a heating block. Centrifuge for 1 min at 12,000 rpm and apply the supernatant as sample.

Note: Repeat the centrifugation step in case of strong turbidity (if necessary).

The RIDA®GENE PVL assay contains an Internal Control DNA (ICD), which can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as a PCR inhibition control.

If the ICD is used only as a PCR inhibition control, 1µl of the ICD should be added to the Master-Mix (see Tab.3).

If the ICD is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the ICD has to be added during extraction procedure. The ICD should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen.

8.3 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and negative control must be included in each assay run.
We recommend to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab.2, Tab.3). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the PCR Water and the ICD before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab.2: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 µl
	Total	**20.0 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab.3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit Code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

8.4 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl PCR Water as negative control to the pre-pipetted Master-Mix.

Note: *If the ICD is used as extraction control for the sample preparation procedure* ***and*** *as PCR inhibition control, we recommend to add 1 µl of the ICD to the negative control PCR-Mix.*

Sample: Add 5 µl DNA-extract to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: *If the ICD is used as extraction control for the sample preparation procedure* ***and*** *as PCR inhibition control, we recommend to add 1 µl of the ICD to the positive control PCR-Mix.*

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument Set-up (see Tab.4, Tab.5).

8.5 PCR Instrument Set-up

Tab.4: Real-time PCR profile for LightCycler® 480II, LightCycler® 2.0, SmartCycler® and Rotor-Gene Q

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	10 sec, 95 °C
Annealing / Extension	15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: *Annealing and Extension occur in the same step.*

Note: Check that the Manual Tresh Flour Units for Channel 1 is set on 30.0 and for Channel 2 is set on 5.0 on the SmartCycler® (Cepheid).

Tab.5: Real-time PCR profile for Mx3000P, Mx3005P, ABI7500 and m2000rt

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	15 sec, 95 °C
Annealing / Extension	30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: *Annealing and Extension occur in the same step.*

8.6 Detection Channel Set-up

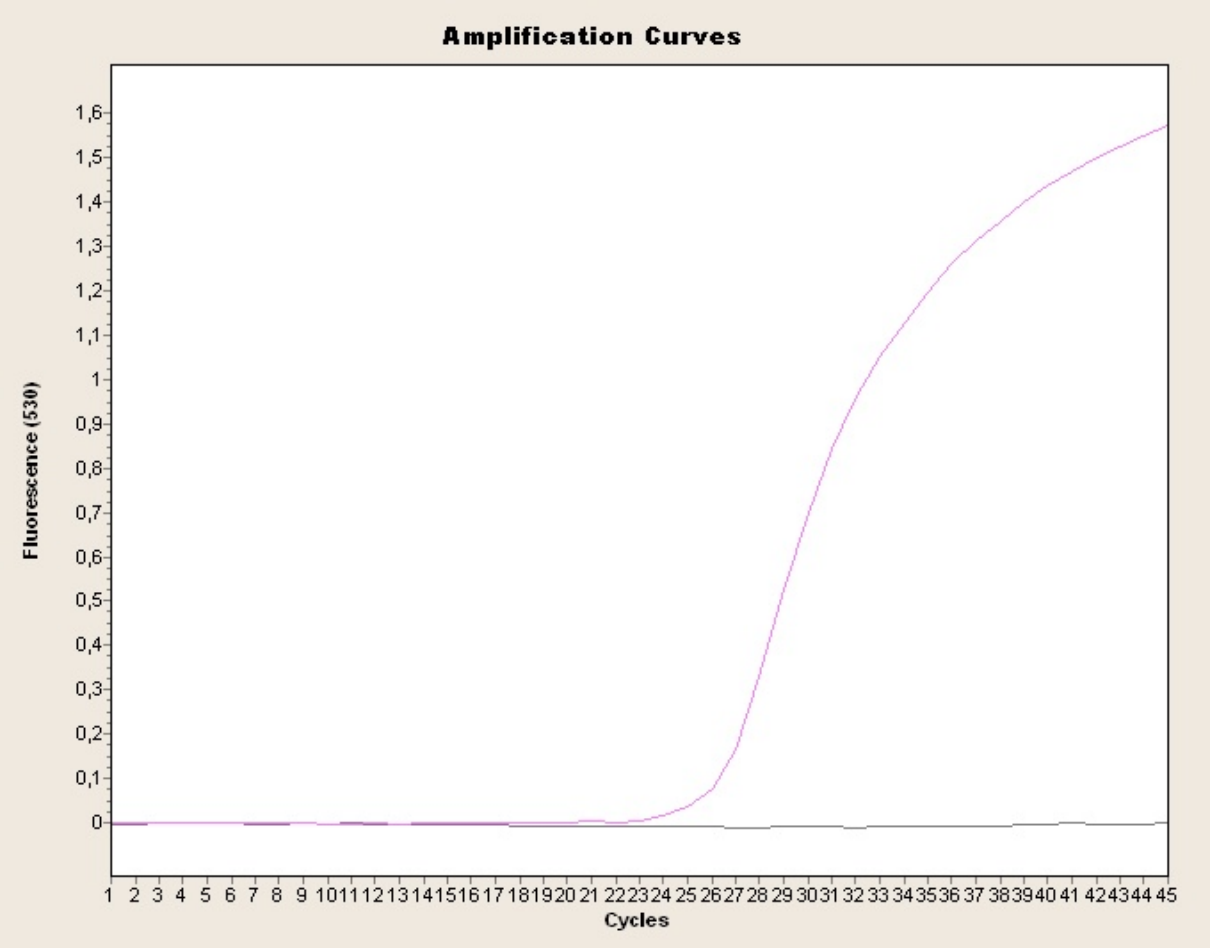
Tab.6: Selection of appropriate detection channels

Real-time PCR Instrument	Detection	Detection Channel	Dark-Quencher	Note
Roche LightCycler® 2.0	PVL	530	+	**RIDA®GENE Color Compensation Kit II (PG0002) is required**
	ICD	560	+	
Roche LightCycler® 480II	PVL	465/510	+	**Color Compensation is not required**
	ICD	533/580	+	
Cepheid SmartCycler®	PVL	Channel 1	+	**Check that the Manual Tresh Flour Units for Channel 1 is set on 30.0 and for Channel 2 is set on 5.0**
	ICD	Channel 2	+	
ABI 7500	PVL	FAM	none	**Check that passive reference option ROX is none**
	ICD	VIC	none	
Abbott m2000rt	PVL	FAM	none	**-**
	ICD	VIC	none	
Stratagene Mx3000P/ Mx3005P	PVL	FAM	+	**Check that reference dye is none**
	ICD	HEX	+	
Qiagen Rotor-Gene Q	PVL	Green	+	**-**
	ICD	Yellow	+	

9. Result interpretation

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer's instructions. Positive and negative controls have to show correct results (see Fig.1).
The positive control has a concentration of 10^3 copies / µl. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Fig.1: Correct run of the positive and negative control (PVL-gene) on the LightCycler® 2.0



The result interpretation is done according to Table 7.

Tab.7: Sample interpretation

PVL-gene	ICD	Result
positive	**positive**	**PVL-gene detected**
positive	negative	**PVL-gene detected**
negative	**positive**	**Target gene is not detected**
negative	negative	**Invalid**

The PVL-gene is detected, if the sample DNA and the Internal Control DNA (ICD) show an amplification signal in the detection system.

The PVL-gene is also detected, if the sample DNA shows an amplification signal but none for the Internal Control DNA (ICD) in the detection system. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA (ICD).

The PVL-gene is not detected, if the sample DNA shows no amplification signal, but an amplification signal for the Internal Control DNA (ICD) in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA (ICD).

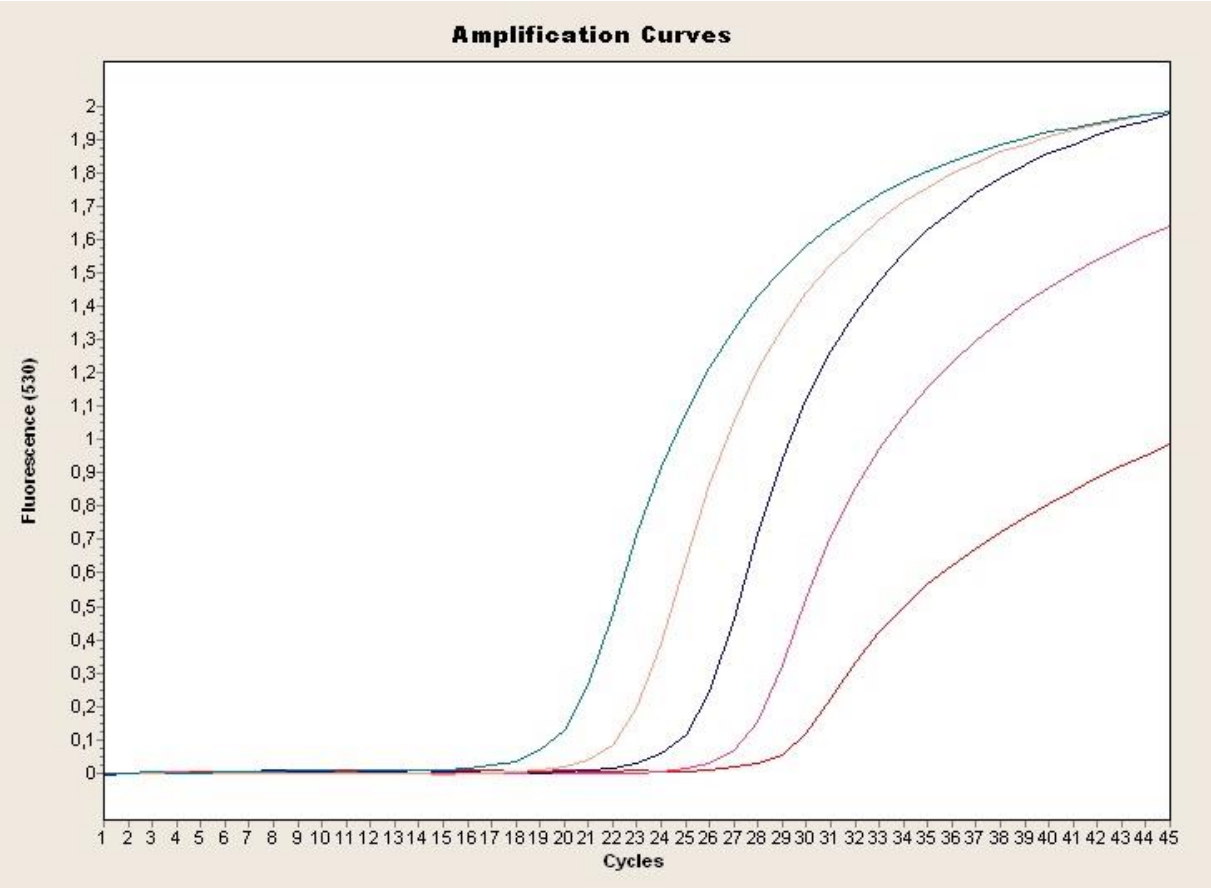
A sample is invalid, if the sample DNA and Internal Control DNA (ICD) show no amplification signal in the detection system. The sample contains a PCR inhibitor. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

10. Test characteristics

10.1 Analytical sensitivity

The RIDA®GENE PVL real-time PCR has a detection limit of ≤ 5 DNA copies per reaction (see Fig.2).

Fig.2: Dilution series PVL-gene (10^5 - 10^1 DNA Copies / µl) on the LightCycler® 2.0



The detection limit of the whole procedure depends on the sample matrix, DNA-extraction and DNA-concentration.

10.2 Analytical specificity

The RIDA®GENE PVL real-time PCR is specific for the PVL-gene (Panton-Valentine leukocidin) from cultures. No cross-reaction could be detected for the following species (see Tab.8):

Tab.8: Cross-reactivity testing

Non-staphylococcal species (number tested)					
Arcobacter butzleri (1)	-	*Citrobacter freundii (1)*	-	*Proteus vulgaris (1)*	-
Aeromonas hydrophila (1)	-	*Clostridium difficile (1)*	-	*Pseudomonas aeruginosa (1)*	-
Bacillus cereus (1)	-	*Clostridium perfringens (1)*	-	*Salmonella enteritidis (1)*	-
Bacteroides fragilis (1)	-	*Clostridium sordellii (1)*	-	*Salmonella typhimurium (1)*	-
Campylobacter coli (1)	-	Enteropathogenic *E. coli (1)*	-	*Serratia liquefaciens (1)*	-
Campylobacter jejuni (1)	-	Enterotoxigenic *E. coli (1)*	-	*Shigella flexneri (1)*	-
Campylobacter fetus (1)	-	Shiga toxin producing *E. coli (1)*	-	*Staphylococcus epidermidis (1)*	-
Campylobacter lari (1)	-	*Enterobacter cloacae (1)*	-	*Vibrio parahaemolyticus (1)*	-
Campylobacter upsaliensis (1)	-	*Enterococcus faecalis (1)*	-	*Yersinia enterocolitica (1)*	-
Candida albicans (1)	-	*Klebsiella oxytoca (1)*	-		
Methicillin-sensitive coagluase-negative *Staphylococci* (number tested)					
S. epidermis (4)	-	*S. warneri (4)*	-	*S. lugudensis (2)*	-
S. hominis (4)	-	*S. pettenkoferi (1)*	-		
Methicillin-resistant coagluase-negative *Staphylococci* (number tested)					
S. haemolyticus (2)	-	*S. epidermis (13)*	-	*S. capitis* (2)	-
Borderline oxacillin-resistant *Staphylococcus aureus* (1)					-
Methicillin-sensible *Staphylococcus aureus* (9)					-

11. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for cultures.
3. Inappropriate specimen collection, transport, storage and processing or a viral load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants resulting in a false negative result with the RIDA®GENE PVL assay.
6. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of PVL DNA since the RIDA®GENE PVL assay simultaneously detects the PVL gene (IukF-PV).
7. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible
8. A RIDA®GENE PVL positive result does not necessarily indicate treatment eradication failure since DNA may persists. A negative result following a previously positive test result may indicate treatment eradication success or may occur due to intermittent shedding.

12. Literature

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