VELLAP

Instructions for use

PVL

This qualitative lateral flow assay is a chromatographic immunoassay for the detection of Panton Valentine Leukocidin (PVL, the gene product of *lukS-PV/ lukF-PV*) in clonal cultures of *Staphylococcus aureus*.

VELLAP PVL Instructions for Use - Revision EN-1.3.5.1946-3 (08.09.2025)

Product information

This qualitative lateral flow assay is a chromatographic immunoassay for the detection of Panton Valentine Leukocidin (PVL, the gene product of *lukS-PV/ lukF-PV*) [1, 2] in clonal cultures of *Staphylococcus aureus*. This product is intended exclusively for research purposes (Research Use Only – RUO).

It is not intended for diagnostic, therapeutic or clinical use and must not be used for patient care or therapeutic decision making and it is not a medical device within the meaning of Regulation (EU) 2017/746.

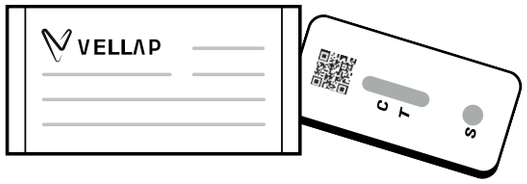
REF	Σ
2.13.6.29	10

Safety note

Please read the instructions for use carefully before you perform the test and keep them in a safe place for future reference.

Components of the test kit

Foil pouch with test cassette



Disposable ampoule

Tube

Transferpipette

Material needed but not provided:

- Colombia Blood Agar or other appropriate growth media for *S. aureus*
- Inoculation loop (1 µl)
- Vortexer
- Incubator
- Table-top centrifuge (e.g. VWR Mini Star Silverline or equivalent)
- Timer (or alternatively the integrated Timer in the VELLAP Scan App)

Please make sure that:

- the components are undamaged
- the shelf life has not been exceeded
- the test is not used as the sole diagnostic method

Table of contents

1. Purpose of the application

This qualitative lateral flow assay is a chromatographic immunoassay for the detection of Panton Valentine Leukocidin (PVL, the gene product of *lukS-PV/ lukF-PV*) [1, 2] in cultures of *Staphylococcus aureus*.

The assay is intended strictly as a research or epidemiological tool for screening monoclonal *S .aureus* cultures for the presence of PVL. It is not intended to diagnose community-associated MRSA infections or to solely guide therapy. Therapeutic decisions are to be based on clinical presentations and need to consider susceptibility test results, indications and contraindications.

This product is intended exclusively for research purposes (Research Use Only – RUO). It is not intended for diagnostic, therapeutic or clinical use and must not be used for patient care or decision making.

2. Medical background

Panton Valentine Leukocidin (PVL) is a virulence factor of *S. aureus*, which is associated with chronic/recurrent skin and soft tissue infections (SSTI) [3-7] as well as with necrotizing pneumonia [8, 9]. PVL genes, *lukS-PV* and *lukF-PV*, are phage borne [10, 11], and since they are mobile, they can be found in different, unrelated lineages of *S. aureus* including both, methicillin-susceptible and -resistant strains [12, 13]. PVL consists of two distinct components, which form polymeric pores in the membranes of white blood cells that lead to cell death [2, 14].

PVL detection is currently performed using molecular methods, that usually are restricted to specialized laboratories with dedicated hardware and trained personnel, and that require complex sample preparation. However, patients with SSTI usually present to family physicians and primary care centres that may not have ready access to such laboratory facilities. These cases remain undiagnosed and thus they will often not adequately be treated. This assay aims mainly on basic laboratories that can perform culture and susceptibility tests, but that are not equipped to perform molecular studies. It is designed to detect the expression of PVL in clonal overnight cultures of *S. aureus* on standard growth media.

3. Safety instructions and precautions

- This product is intended exclusively for research purposes (Research Use Only – RUO). It is not intended for diagnostic, therapeutic or clinical use and must not be used for patient care or decision making.
- Read and understand the entire test instructions before testing.
- Make sure all components are intact.
- Do not use the test after the expiry date.
- *S. aureus* is generally to be handled as a biosafety level II agent. Check local regulations, safety and security rules.
- All specimens and cultures as well as used test units, inoculation loops and rubber gloves are to be considered hazardous and must finally be disposed of as infectious waste.
- Dispose of and autoclave agar plates, colony material, test devices, used gloves and consumables.
- Do not pipette reagents by mouth. Do not smoke, eat nor drink while handling specimens and test kits. Follow the best precautions against microbiological risks during the test execution. Wear protective clothing such as a laboratory apron, disposable gloves and eye protection when specimens are examined.
- Humidity and temperature may adversely influence the test and can lead to false test results.
- Do not use if the pouch of the test cassette is damaged or if buffer looks turbid.
- Only use each test cartridge once.
- The test involves products of animal origin. Even certificates of origin and / or the health status of the animals can not completely guarantee the absence of communicable disease-causing ingredients. It therefore is advised to consider these products as potentially infectious and to take precautions.

- Avoid cross-contamination of samples by using a new sample assessor for each sample collected.
- Do not inject the solution into the reaction window.
- Do not touch the reaction window of the test cassette to avoid contamination.
- Do not replace or mix reagents from different lots.
- The test should not be used for a direct detection of PVL from patient samples. It has been designed for the detection of PVL from monoclonal bacterial culture samples.
- The test was designed using cultures harvested from Colombia Blood Agar.
- Contaminated cultures, old cultures and poor/nutrient-deficient growth media or the use of growth media that contain antibiotics might yield false-negative results.
- The manufacturer accepts no liability for the use of this product for diagnostic or therapeutic purposes.
- Clinics or research institutions are responsible for validating the test themselves if they wish to use it internally.

4. Test principle

The assay is a membrane-based immunoassay. The test device contains PVL-specific IgG antibodies coated to particles and other PVL-specific IgG antibodies coated on the membrane. During testing, the molecules from the sample react with the antibodies coated to the particles. The mixture migrates by capillary force to react with capture antibodies on the membrane and to generate a coloured test line. Its presence in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured control line will always appear in the control line region, indicating that a proper sample buffer volume has been added moistening the membrane.

5. Contents and storage

Each test contains the following reagents:

- 0.1 µg gold-labeled anti-PVL antibody
- 0.5 µg membrane-fixed anti-PVL antibody
- 2.7 mg buffer salt and non-reactive components

Materials supplied per test procedure:

- 1 test cassette in foil pouch with desiccant in test housing
- Disposable ampoule with 0.3 ml buffer solution
- 1.5 ml safe-lock tubes
- Transfer pipette

Additional materials required per test procedure:

- Colombia Blood Agar or other suitable growth media
- Inoculation loop (1 µl)
- Vortexer
- Incubator
- Table-top centrifuge (e.g. VWR Mini Star Silverline or equivalent)
- Timer (or alternatively the integrated Timer in the VELLAP Scan App)

Shelf life and storage

- Store at 2-30°C until the stated expiry date.
- The ambient temperature for performing the test is 15-25°C.
- After opening the foil pouch, the test must be performed within 1 hour.
- The test is designed for single use.
- Do not freeze the test or the components.

6. Handling and disposal

All waste must be disposed of in accordance with applicable local regulations and instructions as well as the guidelines of your facility. The samples must be regarded as potentially infectious. The usual precautionary measures according to the generally applicable laboratory guidelines must be observed during handling.

7. Performance claim

In two independent studies [15, 16], the VELLAP PVL test was comparatively evaluated against the detection of the PVL gene using a gene microarray. Resulting Sensitivity of 98.2 % and Specificity of 99.2 %.

		PVL status by gene detection (using microarray)		
		Positive (+)	Negative (-)	Total
VELLAP PVL	Positive (+)	92	122	214
	Negative (-)	1	1	2
	Total	92	123	216

8. S. aureus cultivation

The test must be performed with culture material of clonal isolates of *Staphylococcus aureus* obtained from clinical routine procedures. The use of fresh (overnight to 24 hours) and pure cultures is highly recommended. Isolates from following diagnoses are generally suspect to express PVL although PVL-rates might vary in different geographic regions:

- furunculosis or carbuncles cutaneous abscesses
- other conspicuous or severe skin and soft tissue infections such as mastitis or necrotising fasciitis
- chronically purulent and painful “spider bites”, especially in cases with travel history
- recurrent or chronic skin- and soft tissue infections
- necrotising community-acquired pneumonia, including cases associated with influenza
- suspected or proven staphylococcal superinfection of COVID-19 coronavirus pulmonary disease

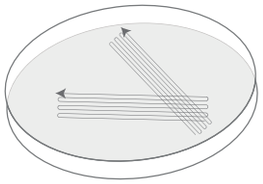
Diabetic foot ulcers are usually not associated with PVL, although PVL might be detected also in such isolates in geographic regions where PVL rates are high. Any *S. aureus* strain might be associated with that condition regardless of virulence factors or PVL status as the clinical course is determined by host factors – such as poor circulation, low oxygen concentration, tissue necrosis, and poor immunological defences. PVL is not associated with bacteraemia/sepsis/endocarditis but might be detected in blood culture isolates in regions with high prevalence.

9. Preparation & realisation

Check the packaging and the components of the kit for damage. Do not use the test kit if damage indicates reduced performance.

1. Read the instructions for use completely. Unpack all kit components.

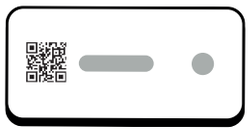
2. Make sure that all components are undamaged and that the shelf life has not been exceeded.



3. Start with culturing of the S. aureus samples (over night) before open the foil pouch including the test cassette.

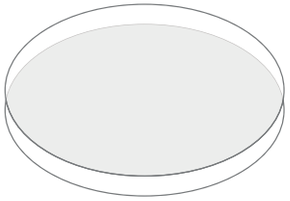
4. Do not open the pouch when not running the test within the next hour.

5. Open the foil pouch at the tear line and remove the test cassette. Place the test cassette on a level and clean surface.



6. Make sure that the test strip is undamaged.

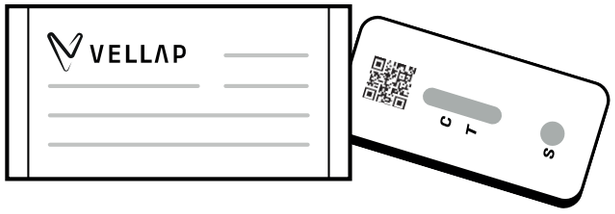
9.1 Sample preparation



1. Sample preparation

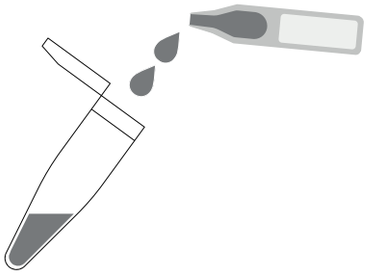
Culture/Subculture the *S. aureus* strain or isolate on a suitable growth medium (see above). Incubate the plate overnight or up to 24 hrs at 37°C ± 2°C.

9.2 Test preparation



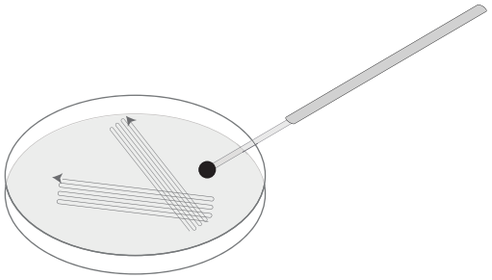
2. Open the pouch

Remove the test device from the foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.



3. Preparation sample extraction

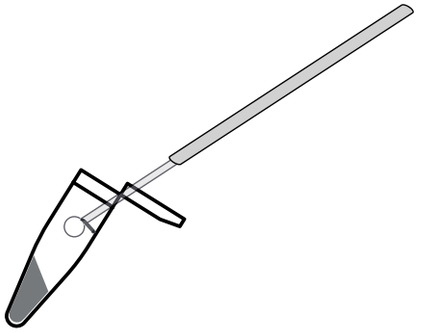
Fill the entire content of the disposable ampule with 0.3 ml buffer solution into an empty 1.5 ml safe-lock tube.



4. Sample collection

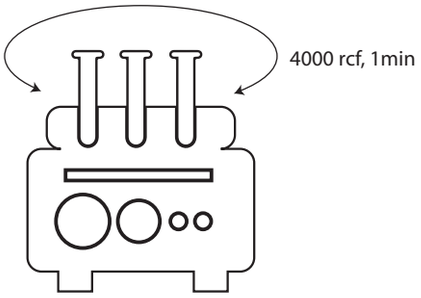
Harvest one inoculation loop (ca. 1 µl) full of culture material.

9.3 Test procedure



5. Sample extraction

Rub the culture material on the tube wall just above the buffer front. Tilt the tube slightly and rinse liquid over the culture material until almost of the sample material has detached from the inoculation loop. Vortex for 15 - 30 sec until cell suspension appears homogenous.



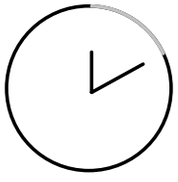
6. Centrifuge

Shortly centrifuge the buffer with the suspension in order to sediment the cells (1 min at 4.000 rcf).

7. Sample application

Pipet 100 µl (or use 3 drops with the Transfer pipette) of the supernatant onto the sample well (S) of the test device.

Do not pippette the pellet (cells) on the assay.



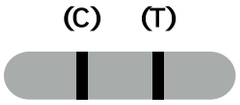
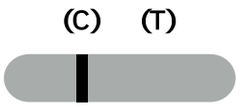
8. Incubation

Incubate 10 min at room temperature.

9.4 Interpretation of results

If no control line appears, the test result is not valid. The test must be repeated with another test cassette.

- The control line is only used to verify the function of the test cassette.
- Depending on the nature of the sample and the PVL concentration, the test line may appear with varying intensity and speed. These factors should not be used for evaluation.
- The test line is generally weaker than the control line and even the finest recognisable line in the test line area is to be evaluated as a positive test result.
- Bright and indirect light is best suited for reading the test. Shadows on the reading window should be avoided.
- Only analyse the result after 10 minutes and not after more than 20 minutes.

	***Positive result:*** Test and Control-line are visible	PVL was detected in the culture sample. Do not use the test result only as the sole basis for treatment decisions.
	***Negative result:*** Only Control-line is visible	A negative result indicates a low probability that the culture produces PVL.
	***Invalid result:*** No Control-line is visible	The test cannot be evaluated. Please repeat the test with another test cartridge.

9.5 Documentation with VELLAP SCAN

This product can be used particularly conveniently with the **VELLAP SCAN** mobile app.

The app is available to you free of charge and can be installed using the adjacent **QR code,** which automatically redirects you to the iOS App Store or the Google Play Store, depending on your end device.

You can use the app on any number of devices and can also work conveniently in a team using the group function.

With the multi-timer function of the **VELLAP SCAN** app, you can easily keep an eye on the test run times thanks to the individual labelling of the test cassettes, even if you are carrying out many tests in parallel.

Based on a high-performance AI specially trained for the VELLAP tests, the **VELLAP SCAN** app detect the test line as precisely as a strip test analyser.

Please note that the app is only a tool for documenting the test results.

The purpose of the test remains unaffected.

10. Symbols

	Sufficient for „n“ Tests		Keep away from sunlight
	Temperature		If the package is damaged, do not use
	Do not reuse		Store in a dry place
	Manufacturer		Product expiry date
REF	Article number	LOT	Batch number
RUO	Research Use Only		Instruction

11. Literature

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This product was designed, developed and manufactured in Germany.

Made in Germany

senova IMMUNOASSAY SYSTEMS

VELLAP

FLOWIFY AI