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suPARnostic® Quick Triage test for aLF Reader

Instructions For Use

Test Device

REF A003

ViroGates A/S
Banevaenget 13
Birkeroed 3460, Denmark
Tel: +45 2113 1336
www.virogates.com

CE IVD

suPARnostic®

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Refer to the webpage http://www.virogates.com for instructions for other biochemistry analysers and languages. Alternatively, contact your local distributor for instructions in your language.

INTENDED PURPOSE

For in vitro diagnostic use.
The suPARnostic® Quick Triage is an in vitro diagnostic assay used to determine the soluble urokinase Plasminogen Activator Receptor (suPAR) in human K2-EDTA- and lithium heparin plasma. The suPARnostic® Quick Triage test is measured on a Color metric Reader.
The suPARnostic® Quick Triage is a quantitative test measuring the suPAR level in ng/mL and is intended for use as an aid in the detection and evaluation of inflammatory disorders and immune activation.

INTENDED USER AND PATIENT

For professional use.
Typical users are laboratory technicians in central laboratories.
The typical patients are present in the Emergency Departments (ED) or the Intensive Care Units (ICU).

<u>Acute Medicine</u>

For unselected acute care patients, the suPARnostic® Quick Triage is used to identify inflammation and immune activation to aid in determining the risk of 30 days mortality to support triage decisions, in conjunction with clinical findings and the results of other laboratory tests.

COVID-19

In patients with confirmed COVID-19 virus, the suPARnostic® Quick Triage is used to identify inflammation and immune activation to aid in determining the risk of respiratory failure and the need for mechanical ventilation in conjunction with clinical findings and the results of other laboratory tests.

suPAR IS A MARKER OF DISEASE PROGRESSION

suPAR is the soluble form of urokinase Plasminogen Activator Receptor (uPAR). The amount of suPAR is a measure of immune activation and inflammation.[1] suPAR is a biomarker increased by disease presence and severity.

In unselected ED patients, suPAR has a high negative predictive value for ruling out disease progression[2]. This means that patients with a low (<4 ng/mL) suPAR level have a good prognosis and a low risk of readmission and mortality[3], supporting the decision to discharge the patient. Conversely, a high suPAR level (>6 ng/ml) is a strong measure of chronic inflammation and the underlying risk of adverse outcomes, including short-term mortality (in hospital, 30 days, or 90 days)[2] supporting the decision of further examination of the patient.

Using suPAR in clinical routine adds significant additional knowledge to the standard assessment based on early warning score systems and standard parameters in pre-admission of acute medical patients. Hence, suPAR is a broadly applicable biomarker, e.g., in the ED, especially concerning discharge decisions of patients and identifying non-diagnosed inflammatory diseases.

A cluster-randomised interventional study showed that up- or down-triaging patients based on suPAR levels increased the number of patients discharged (low risk) by 34%[4] and reduced hospital bed days[5].

In patients with confirmed COVID-19, suPAR levels below 4 ng/mL suggest a low risk of developing respiratory failure and supports the decision of discharge for home quarantine.[6] COVID-19 patients with a suPAR level above 6 ng/ml have high risk of developing respiratory failure. This risk can be reduced by treatment with Anakinra[16].

TEST PRINCIPLES

The suPARnostic® Quick Triage is a lateral flow immunoassay. The device utilizes monoclonal rat and gold-conjugated mouse antibodies against human suPAR to measure the plasma suPAR level. The K2-EDTA and Lithium Heparin -plasma is mixed with running buffer and applied to the suPARnostic® Quick Triage device. During the 20 minutes of incubation, the plasma sample reacts with gold-conjugated anti-suPAR antibodies and migrates through the nitrocellulose membrane. The gold-conjugate containing sample suPAR is bound by a capture suPAR antibody at the Test line.

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In contrast, the non-suPAR bound antibody is captured by the Control line (anti-mouse antibody). The suPARnostic® Quick Triage is calibrated against an internal control. No international standard has been established.

REAGENTS AND MATERIALS

Reagents Provided

This kit contains reagents sufficient to perform 25 test devices.

- Lateral Flow Devices: each in an aluminum pouch with a desiccant sachet. Quantity: 25 devices. Preparation: Ready to use.
- Assay Running Buffer: PBS buffer, pH 7.2, with proprietary additives and 0.05% Bronidox® as a preservative.
  Quantity: 3.5 mL. Preparation: Ready to use.
- Instruction for use.
- Barcodes to upload the methods.

Material required but not provided

- Adjustable pipette with tips, 10– 100 µL.
- Clock, timer, or stopwatch.
- Disposable gloves.
- aLF Reader (#9002770).
- Eppendorf tube or other mixing tubes.

PRECAUTIONS

For professional laboratory use.

For in vitro diagnostic use. Exercise the standard precautions required for handling all laboratory reagents. Disposal of all waste material should follow local guidelines. The safety data sheet is available for professional users upon request.

- Do not use kit components beyond the indicated kit expiration date.
- Do not freeze any of the kit components.
- Do not mix reagents from different kit lots.
- Do not switch caps on reagent containers, as it may cause contamination or mix-up.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Do not mix plasma samples from different patients or blood samplings of the same patient.
- Human samples may be contaminated with infectious agents. Therefore, do not ingest, expose to open wounds, or breathe aerosols.
- Wear protective gloves and dispose of biological samples according to the regulations.
- Be aware of possible dilution of suPAR in the case of a transfusion, infusion or similar.

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- Do not use a device if the pouch is damaged or opened.

STORAGE AND HANDLING

Test device

The test devices must be stored in the sealed foil pouches.

Store kit components at 18 – 24°C.

The test devices and running buffer may be used until the date printed on the pouch or bottle.

Tightly close the cap of the running buffer after each use.

IMPORTANT: The test devices must be used immediately after opening the pouch. It cannot be stored for later use.

SAMPLE COLLECTION AND PREPARATION

Sample type: Fresh plasma sample

Sample requirement: 10 µL

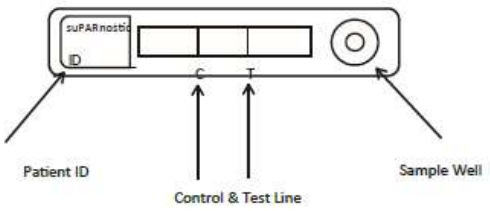
To prepare plasma samples, draw whole blood into a blood collection tube containing either K2-EDTA or Lithium Heparin anticoagulant. Then, centrifuge the blood at 3,000 x g for 1-10 minutes or until blood cells and plasma have separated.

Transfer and store plasma samples in separate marked tubes and date and identify each sample.

Important: Only plasma samples before freezing can be used with the suPARnostic® Quick Triage kit.

NOTE Grossly hemolysed, lipemic, or microbiologically contaminated samples should not be used. Samples with abnormally elevated haemoglobin levels or bilirubin may interfere with assay performance and sensitivity.

DEVICE DESCRIPTION



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ASSAY PROCEDURE

NOTE: It is critical that the volumes pipetted and the incubation time is followed precisely as described in the procedure.

Two measuring methods are offered for each batch of suPARnostic® Quick Triage tests.
The *suPARnosticQT* method starts measuring the suPAR level when the' forward' button on the reader is pressed.
The *suPARnosticQT20* method measures the suPAR level after 20 minutes of incubation time. Allowing the user to insert the device in the reader during the incubation and ensure the correct incubation time.

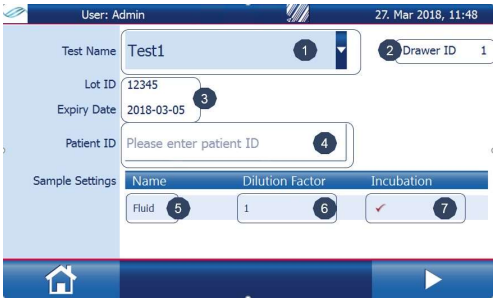
suPARnosticQT – for manual reading	suPARnosticQT20 – for automated reading
1. Transfer 100 µL of running buffer to an empty tube.	
2. Transfer 10 µL of plasma sample to the tube containing 100 µL of running buffer. Vortex the mixture or use the pipet to mix up and down.	
3. Transfer 60 µL of the diluted sample to the well of the suPARnostic® Quick Triage test device.	
4. Incubate the test device for 20 minutes on the table. Scan the *suPARnosticQT* method barcode and insert the test device into the Reader *(If the user is NOT present during the incubation, it is recommended to use the suPARnosticQT20 for automated reading of the result).*	4. Scan the *suPARnosticQT20* method barcode. Insert the device in the aLF Reader for incubation and touch "forward" to activate the 20-minute incubation.
5. Press "forward" to read the test device after precisely 20 minutes.	5. The Reader reads the suPAR level automatically after 20 minutes.

RUNNING A TEST – aLF Reader

Turn on the Reader.

- Touch the "New Test" field on the touchscreen display to start a new test.
- Scan the *suPARnosticQT* or the *suPARnosticQT20* barcode using the internal 2D-barcode scanner on the aLF Reader.
  NOTE: keep the barcode at a vertical angle.

- The test screen below will appear. The fields: Test name (1), Drawer ID (2), Lot ID and expiry date (3), and Sample Settings (5-7) will automatically appear on the screen.



- Scan the 2D barcode with the Patient ID or write the Patient ID manually. Open the tray on the right side of the reader. Insert the device with the patient ID to the left and the sample well to the right after adding the patient sample.
- Touch the "forward" button to proceed and confirm that the cassette has been inserted in the correct orientation.
- The suPAR result will be displayed in ng/mL.
- The suPAR value should be within the range of 2-15 ng/mL. If the result is out of range, it will be shown as < 2.0 ng/mL or >15 ng/mL. If the display shows INVALID, an error has occurred during the measurement. Re-run the sample, and if the result is INVALID again, check the extensive instruction for the aLF reader on the internet, or contact ViroGates for support by phone number +45 2113 1336 or by email to info@virogates.com

CALCULATION and CALIBRATION

The suPARnostic® Quick Triage test device must be used with the aLF reader to give correct values. The user cannot evaluate the results by visually inspecting the suPARnostic® Quick Triage test device. The aLF Reader automatedly performs the calculation of suPAR levels. The aLF reader scans the test- and control line and determines the intensity of the lines. The calculation to estimate the suPAR value is based on the test line. The aLF Reader uses a batch-specific method for each batch of Quick Triage test devices for the calculation. The batch-specific method is a QR code in the test device kit. The method contains a calibration curve the reader uses to convert the T-Line's intensity to ng/mL suPAR.
The mathematical calculation is made with a linear curve based on 6 reference samples with known concentrations and a buffer.

QUALITY CONTROL

The suPARnostic® Quick Triage test uses the C-Line as the internal quality control. The result is faulty if the C-line does not appear on the test device after an otherwise successful run of the plasma sample.

The aLF Reader will automatically display if any error has occurred during measurements. The reader has an internal quality control run every time the reader is turned on.

INTERPRETATION OF RESULTS

suPAR levels and cut-offs

Acute care medical patients and risk of 90-day mortality

The cut-offs for interpreting results from acute care patients were established based on suPAR baseline measurements of 990 patients attending the ED in a Spanish multicenter trial.[5] The median age was 68 years (53-81), 50.8% were men, median suPAR was 3.8 ng/mL (Interquartile range 2.8-6.0). In total, 47 died during the 90-day follow-up. Of the 990 patients, 520 (52.5%) had suPAR below 4.0 ng/mL. Patients with suPAR <4.0 ng/mL had a low risk of 90-day mortality (N=5, 0.96%), resulting in a negative predictive value (NPV) of 99.0%, a sensitivity of 89.4% and a specificity of 54.6%. In patients with suPAR >6.0 ng/mL (N=245 (24.8%), 33 patients died during the 90-day follow-up (13.5%), resulting in a positive predictive value (PPV) of 13.5%, a sensitivity of 70.2%, and a specificity of 77.5%.

	90 Days follow-up				
	Died	Survived	Total	PPV	NPV
High risk (suPAR >6.0 ng/mL)	33	212	245	13.5%	
Medium Risk (suPAR 4.0–6.0 ng/mL)	9	216	225		
Low risk (suPAR <4.0 ng/mL)	5	515	520		99.0%
Total	47	943	990		
Sensitivity/specificity (<4.0 ng/mL)	89.4%	54.6%			
Sensitivity/specificity (>6.0 ng/mL)	70.2%	77.5%			

Table 1: 90-day mortality according to suPAR cut-offs in Spanish multicenter study.

COVID-19 and the risk of respiratory failure

For patients who tested positive for the COVID-19 virus, suPAR baseline measurements were taken within 48 hours after patients presented at the hospital[6]. Respiratory failure was defined as the need for mechanical ventilation within 2 weeks. The study included 57 patients, of whom 21 developed respiratory failure. None of the patients with suPAR below 4.0 ng/mL developed respiratory failure resulting in an NPV of 100%, a sensitivity of 100% and a specificity of 36.1%. Of the 21 patients who developed respiratory failure, 18 had baseline suPAR levels above 6.0 ng/mL resulting in a PPV of 85.7%, a sensitivity of 85.7%, and a specificity of 81.3%.

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suPAR level	Interpretation, ED and COVID-19
<4.0 ng/mL	**Low Risk** Supports the decision of discharge. The underlying health condition is good, and the prognosis for survival is high. Low risk of respiratory failure and need of mechanical ventilation in patients with COVID-19.
4.0-6.0 ng/mL	**Medium Risk** Some disease activity or co-morbidity is present. Some readmissions and mortality are expected after six months of follow-up. Medium risk of respiratory failure and need of mechanical ventilation in patients with COVID-19.
>6.0 ng/mL	**High Risk** Clinical attention is needed - high risk of mortality. Supports the decision of admission and treatment[16)]. High risk of respiratory failure and need of mechanical ventilation in patients with COVID-19.

Table 2: Simplified suPAR clinical decision scheme [6, 11].

EXPECTED VALUES IN HEALTHY INDIVIDUALS

All individuals have a measurable suPAR level. In healthy blood donors (N=9305), the median suPAR level for men aged 18-65 years is 2.2 ng/mL (25-75% interval from 1.8-2.9 ng/mL)[7], for women aged 18-65 years is 2.6 ng/mL (25-75% interval from 2.1-3.2 ng/mL)[7], patients attending ED the suPAR level is around 3.0-6.0 ng/mL[3,4,8]. In patients with severe disease and organ failure, suPAR is often double-digits[9,10]. The higher the level, the higher the risk of disease progression and the worse the prognosis.

CLINICAL PERFORMANCE

Validation of cut-offs

Acute-care medical patients

The clinical validation data are from a prospective observational study of unselected acute medical patients attending the ED at Mikkeli Hospital in Finland.[11] A total of 1747 acute medical patients were included and had suPAR measured using suPARnostic® TurbiLatex. The median age was 70 (IQR: 57-79), and 51.4% were men. Among patients with suPAR below 4.0 ng/mL (N=804, 46.0%), 8 (1.0%) died within 90-day follow-up, resulting in a negative predictive value of 99.0% and a sensitivity of 94.2% and a specificity of 47.9%. Among patients with suPAR above 6.0 ng/mL (N=429, 24.6%), 87 patients (20.3%) died

within 90-day follow-up, resulting in a positive predictive value of 20.1%, a sensitivity of 63.0% and a specificity of 78.7%. Data for 90-day follow-up are shown in Table 3.

	90-day follow-up				
	Died	Survived	Total	PPV	NPV
High risk (suPAR >6.0 ng/mL)	87	342	429	20.3%	
Medium Risk (suPAR 4.0–6.0 ng/mL)	43	471	514		
Low risk (suPAR <4.0 ng/mL)	8	796	804		99.0%
Total	138	1609	1.747		
Sensitivity/specificity (< 4.0 ng/mL)	94.2%	49.5%			
Sensitivity/specificity (> 6.0 ng/mL)	63.0%	78.7%			

Table 3: 90-day mortality in acute medical patients in Finish validation study[11].

COVID-19

The clinical validation data are from a prospective observational study at Mikkeli Central Hospital in Finland using the suPARnostic® TurbiLatex on a Cobas c 501. The study included 100 acute medical patients who tested positive for SARS-CoV-2 at the Emergency Department at Mikkeli Central Hospital in Finland.[12]

Results of suPAR validation for stratification of COVID-19 patients regarding the risk of developing severe respiratory failure and requiring mechanical ventilation are shown in Table 4.

	90-day follow-up				
	Died	Survived	Total	PPV	NPV
High risk (suPAR >6.0 ng/mL)	5	44	49	10.2%	
Medium Risk (suPAR 4.0–6.0 ng/mL)	0	27	27		
Low risk (suPAR <4.0 ng/mL)	0	24	24		100%
Total	5	95	100		
Sensitivity/specificity (< 4.0 ng/mL)	100%	25.3%			
Sensitivity/specificity (> 6.0 ng/mL)	100%	53.7%			

Table 4: 90-day development of respiratory failure in COVID-19 patients according to suPAR cut-offs.

LIMITATIONS

Clinical prognostication must not be based on the results of the suPARnostic® Quick Triage test alone. Instead, the results must be interpreted considering the patient's clinical history and other diagnostic test results.

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ANALYTICAL PERFORMANCE

SAMPLE STABILITY

Samples should preferably be analysed as soon as possible, but K2-EDTA and Lithium Heparin plasma samples are stable for:

- 24 hours at room temperature (20-25°C).
- 3 days at 2-8°C.

Only freshly drawn plasma samples should be used.

REQUIRED TRAINING

To use the suPARnostic® Quick Triage, it is required that the user is fully trained in how to operate the aLF Reader.

MEASURING RANGE

The measuring range of the suPARnostic® Quick Triage test is 2-15 ng/mL.
It is not recommended to dilute samples with the results above the measuring range.

ANALYTICAL LIMITS

Limit of Blank (LoB) shows the variation of a blank sample (buffer only).
Limit of Detection (LoD) is the lowest possible detection of suPAR that is not a blank sample.
Limit of Quantification (LoQ) is set to be the sample with the lowest concentration in the range 0-2 ng/mL to have a CV% that does not exceed 25%.
The highest value of LoB. LoD. And LoQ from 3 batch validations is presented in the table below.

	LoB	LoD	LoQ
Plasma suPAR (ng/mL)	0.4 ng/mL	1.0 ng/mL	2.0 ng/mL

LoB and LoD were established following CLSI EP17[13]

INTERFERENCE

The substances listed below were tested for interference with the suPARnostic® Quick Triage test. None of the tested substances interfered with the performance of the test.

Substance:	Concentration:
Bilirubin	0.10 – 0.50 mmol/L
Haemoglobin	0.00 – 0.94 mmol/L
Triglycerides	0.00 – 23 mmol/L

Rheumatoid factors:
Samples from 16 patients with increased rheumatoid factor concentrations (0-1600 kIU/L) were analyzed. No significant correlation to rheumatic factors (R^2=0.33) was observed.

LINEARITY

suPARnostic® Quick Triage test is linear from 2.5 ng/mL to 15.2 ng/mL, within a 7.5% degree of nonlinearity in this interval.

HOOK EFFECT

The suPARnostic® Quick Triage test shows no prozone effect in concentrations below 70 ng/mL.

PRECISION

Intra-serial results are estimated on 5 measurements in one day and provide a Mean, Standard Deviation, and CV%. The inter-serial variation is between 5 days.
The highest CV% from 3 batches are displayed below.

Plasma	Mean suPAR level (ng/mL)	Repeatability (CV) (Within run CV (%))	Between-day precision (CV)
Low	2.0	22%	29%
Low	4.0	23%	20%
Medium	7.4	12%	18%
High	14.0	10%	18%

METHOD COMPARISON

Passing-Bablok correlation toward suPARnostic® ELISA has been performed to estimate the Quick Triage ability to quantify suPAR in patient samples.

Results

Sample type	No. of Pair	Slope	Y-intercept	Passing-Bablok correlation	Range Value
Plasma	60	1.13	-0.39	0.893	1.3-18.7

X = suPARnostic ELISA Y = suPARnostic Quick Triage

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WASTE HANDLING

Discard unused reagents and waste following federal, state, and local regulations.

			
Catalogue no.	LOT No. (Batch No.)	Consult instructions for use	Temperature Limits
			
Do not re-use	Do not use it if the package is damaged or open	Contains sufficient for <n> tests	Use by

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