EXPECTED VALUES

All individuals have a measurable suPAR level, and in healthy blood donors (N=9305) the median suPAR level for men aged 18–65 years old is 2.22 ng/mL (25-75% interval from 1.76–2.90 ng/mL)[2], and for women 18-65 years old 2.56 ng/mL (25-75% interval from 2.05–3.23 ng/mL)[2]. In patients attending emergency departments the suPAR level is around 3-6 ng/ml[3,4,7] and in patients with severe disease and organ failure, suPAR is often in the double-digits[5,6]. The higher the suPAR level, the higher the risk of disease progression and the worse the prognosis.

PERFORMANCE CHARACTERISTICS

Limit of Blank (LOB) - show the variation of a blank sample (buffer only). Highest value from 3 batch validations. Limit of Detection (LOD) - is the lowest possible detection of suPAR that is not a blank sample. Highest value from 3 batch validations.

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%. Highest value from 3 batch validations.

	LOB	LOD	LOQ
X (NG/ML) =	0.4	1.0	2.0

Imprecision & Repeatability

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.

	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
X (NG/ML)=	2.0	4.0	7.4	14.0
WITHIN RUN CV (%)	22%	23%	12%	10%
BETWEEN RUN CV (%)	29%	20%	18%	18%

LINEARITY

The Quick Triage analysis of suPAR on the aLF Reader, has been demonstrated to be linear from 2.5 ng/ml to 15.2 ng/ml, within 7.5% degree of nonlinearity in this interval.

HOOK EFFECT

The suPARnostic® Quick Triage shows no prozone effect in concentrations below 70 ng/mL (this is the highest tested suPAR concentration).

ACCURACY (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

WASTE HANDLING

Discard unused reagents and waste in accordance with country, federal, state, and local regulations.

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

REFERENCES

1. www.alf-reader.com
2. Haastrup E, Grau K, Eugen-Olsen J, Thorball C, Kessing LV, Ullum H: Soluble urokinase plasminogen activator re-ceptor as a marker for use of antidepressants. PLoS One 2014, e110555.
3. Raggam RB, Wagner J, Pruller F, Grisold A, Leitner E, Zollner-Schwetz I, et al: Soluble urokinase plasminogen activator receptor predicts mortality in patients with sys-temic inflammatory response syndrome. J Intern Med 2014, 12238.:10.
4. Haupt TH, Petersen J, Ellekilde G, Klausen HH, Thorball CW, Eugen-Olsen J, et al: Plasma suPAR levels are associat-ed with mortality, admission time, and Charlson Comorbid-ity Index in the acutely admitted medical patient: a pro-spective observational study. Crit Care 2012, 16:R130.
5. Koch A, Zimmermann HW, Gassler N, Jochum C, Weiskir-chen R, Bruensing J, et al.: Clinical relevance and cellular source of elevated soluble urokinase plasminogen activator receptor (suPAR) in acute liver failure. Liver Int 2014, 10
6. Donadello K, Scolletta S, Taccone FS, Covajes C, Santono-cito C, Cortes DO, et al: Soluble urokinase-type plasmino-gen activator receptor as a prognostic biomarker in critical-ly ill patients. J Crit Care 2014, 29:144-149.
7. Rasmussen LJH, Ladelund S, Haupt TH, Ellekilde GE, Eugen-Olsen J, Andersen O. Combining National Early Warning Score With Soluble Urokinase Plasminogen Activator Receptor (suPAR) Improves Risk Prediction in Acute Medical Patients: A Registry-Based Cohort Study. Crit Care Med. 2018 Dec;46(12):1961-1968.

ViroGates

Instructions For Use

suPARnostic® Quick Triage for aLF Reader

soluble urokinase Plasminogen Activator Receptor

Test Device

REF **A003**

CE IVD

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

suPARnostic® Quick Triage

This product is protected by one or more US, European, and/or foreign patents.

Refer to www.virogates.com for further instructions and instructions in your local language. Alternatively, contact your local distributor for instructions in your language.

INTENTED USE

For professional use.

The suPARnostic® Quick Triage test is used for determination of soluble urokinase plasminogen activator receptor (suPAR) in human EDTA- and heparin-plasma in ng/ml. The suPARnostic® Quick Triage test is measured on the aLF Reader from Qiagen.[1]

Interpretation of results must be made considering the patient´s clinical history and results of other diagnostic tests if available.

SUMMARY OF suPAR AS A MARKER OF DISEASE PROGNOSIS

suPAR is the soluble form of urokinase plasminogen activator receptor (uPAR). The amount of suPAR is a measure of immune activation and inflammation. suPAR is a non-specific biomarker which is increased by presence of disease. The higher suPAR level, the higher the risk of disease progression and the worse the patient's prognosis.

PRINCIPLES OF ASSAY PROCEDURE

The suPARnostic® Quick Triage is a lateral flow immunoassay. The device utilizes monoclonal rat and gold-conjugated mouse antibodies against human suPAR to give a quantitative measurement of the plasma suPAR level. The EDTA- or heparin-plasma is mixed with running buffer and applied to the suPARnostic® Quick Triage device. During the 20 min. of incubation, the plasma sample reacts with gold-conjugated anti-suPAR antibodies and migrates through the nitrocellulose membrane. Gold-conjugate containing sample suPAR is bound by a capture suPAR antibody at the Test line, while 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 have been established.

REAGENTS AND MATERIALS

Reagents Provided

This kit contains reagents sufficient to perform 25 tests devices.

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

Material required but not provided

- Adjustable pipette with tips, 10 µL – 100 µL.
- Clock, Timer, or stopwatch.
- Disposable gloves.
- aLF Reader (#ESLR12-MB-6401).
- Eppendorf tube or other mixing tube.

REQUIRED TRAINING

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

REAGENT PRECAUTIONS AND RECOMMENDATIONS

- For professional use.
- Do not use kit components beyond the indicated kit expiration date.
- Do not mix reagents from different kit lots.
- Do not freeze any of the kit components.
- 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 from different blood samplings of the same patient.
- Human samples may be contaminated with infectious agents. Do not ingest, expose to open wounds, or breathe aerosols. Wear protective gloves and dispose of biological samples properly.
- Do not use a device if the pouch is damaged or opened in some way.
- Be aware of possible dilution of suPAR in the case of transfusion, infusion, or similar.

REAGENT STORAGE AND HANDLING

Devices must be stored in the sealed foil pouches. Store kit components at 18 – 24°C.
The Devices and running buffer may be used until the date printed on the pouch or bottle.
Tightly close the cap after each use. IMPORTANT: Devices have to be used right after opening the pouch. Cannot be stored for later use.

SPECIMEN COLLECTION

Sample Type	*Sample Requirement*
Plasma Sample	10 µl of Plasma

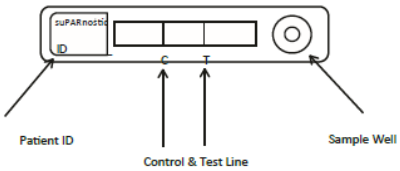
SAMPLE COLLECTION AND STORAGE

Preparation of plasma samples;

1. Whole blood is drawn into a centrifuge tube containing EDTA or heparin anti-coagulant.
2. Centrifuge the blood at 3,000 x g for 1-10 min.
3. Transfer and store plasma samples in separate marked tubes.
4. Date and identify each sample. For long-term storage, keep at -20°C. Avoid freeze/thaw cycles.

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

DEVICE DESCRIPTION



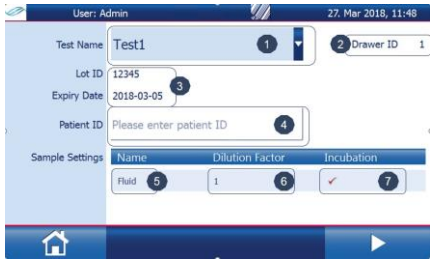
ASSAY PROCEDURE

It is critical that the volumes pipetted and the incubation time is followed precisely as described in this procedure. Two measuring methods are offered for each batch. The ***suPARnosticQT*** method starts measuring the suPAR level when the 'forward' button is pressed.
The ***suPARnosticQT20*** method measure the suPAR level 20 min after the 'forward' button have been pressed. This allows the user to insert the device in the reader during the incubation and ensures the incubation time is correct.

suPARnosticQT	**suPARnosticQT20**
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 device.	
4. Incubate the device for 20 min on the table and insert the device into the aLF Reader before uploading the method. *(If the user is NOT present during the incubation it is recommended to use the suPARnosticQT20).*	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 device with the aLF Reader. Use the designated batch method.	5. aLF Reader reads the device automatically after 20 min.

RUNNING A TEST

1. To start a new test, touch the "New Test" field on the touch screen display.
2. Scan either the *suPARnosticQT* or the *suPARnosticQT20* barcode, using the internal 2D-barcode scanner on the aLF Reader, provide in the kit depending on the preferred measuring method. NOTE: keep the barcode in a vertical angle.
3. Test method name **(1)**, Lot ID **(3),** and Sample Settings **(5-7)** will automatically appear on the screen.
4. Read the 2D-barcode with the Patient ID or write the Patient ID manually.



5. Open tray on right side of the reader. Insert the device with patient ID to the left and the sample well to the right after adding the patient sample.
6. Touch the "forward" button to proceed and confirm that the cassette has been inserted in the correct orientation.
7. The suPAR result will be displayed in ng/ml.
8. The suPAR value should be within the range 2-15 ng/ml. If the result is out of range, it will be shown as < 2.0 ng/ml or >15 ng/ml, and the value cannot be considered as accurate and precise. 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 on the internet, or contact ViroGates for support on phone number +45 2113 1336 or by email to info@virogates.com

QUALITY CONTROL

The suPARnostic® Quick Triage uses the C-Line as the internal quality control. The result is faulty if the C-line does not appear on the device after an otherwise successful run of plasma sample.
The aLF Reader it will automatically display if any error has occurred during measurements. The reader has an internal quality control which is run every time the reader is turned on.

CALCULATION OF RESULTS

The suPARnostic® Quick Triage device has to be used with the aLF reader to give correct values. The user cannot evaluate the results by visually inspecting the Quick Triage device. The aLF Reader automatedly performs the calculation of suPAR levels.
The aLF reader scan the test- and control line and determine 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 included in the test device kit as a QR code. 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 only sample.

LIMITATIONS OF TEST

Clinical diagnosis should not be based on the result of the suPARnostic® Quick Triage test alone. Interpretation of results must be made considering the patient's clinical history and results of other diagnostic tests, if available.

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 mmol/ L
Bilirubin	0.10 – 0.50
Hemoglobin	0.00 – 0.94
Triglycerides	0.00 - 23

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