



Instructions For Use - Short Version

suPARnostic® Quick Triage

Soluble Urokinase Plasminogen Activator Receptor

Test Device

REF A003

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ViroGates A/S
Blokken 45
Birkerød 3460, Denmark
Tel: +45 2113 1336
Skype: Virogates
www.virogates.com



Refer to the webpage www.virogates.com under User Instructions for an extensive instruction in English. Contact your local distributor for instructions in your language.

INTENDED USE FOR PROFESSIONAL USE

The suPARnostic® Quick Triage is used for determination of soluble urokinase plasminogen activator receptor (suPAR) in human EDTA- and heparin-plasma in ng/ml.

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. 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 (LFIA). 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 is 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. 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 since there is not established an international standard.

REAGENTS AND MATERIALS

Reagents Provided

This kit contains reagents sufficient to perform up to 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. Instruction for use of suPARnostic® Quick Test Reader without PC.

Material required but not provided

- Adjustable pipette with tips, 10 µL – 100 µL
- Clock, Timer or stopwatch
- Disposable gloves
- suPARnostic® Quick Test Reader (Lateral Flow Reader) REF - A004
- 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 Quick Test Reader with and without PC.

REAGENT PRECAUTIONS AND RECOMMENDATIONS

- For professional users.
- 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.

STORAGE AND HANDLING

Devices must be stored in the sealed foil pouches.

Store kit components at 18 - 24 °C. Devices may be used until the date printed on the pouch. Do not use devices after expiration date. The Running Buffer may be used until the date printed on the bottle. Tightly close the cap after primary use.

IMPORTANT: Devices have to be used right after opening the pouch. Cannot be stored for later use.

SPECIMEN COLLECTION AND HANDLING

Sample Type	Sample Requirement
Plasma Sample	10 µl of Plasma

SAMPLE COLLECTION AND STORAGE

To prepare plasma samples, whole blood is drawn into a centrifuge tube containing EDTA or heparin anti-coagulant. Centrifuge the blood at 3,000 x g between 1-10 minutes. Transfer and store plasma samples in separate, marked tubes. 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.

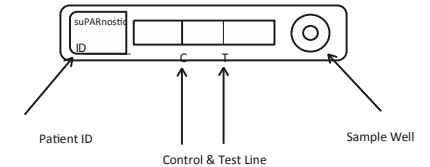
ASSAY PROCEDURE

suPARnostic® Quick Triage (Code No. A003)

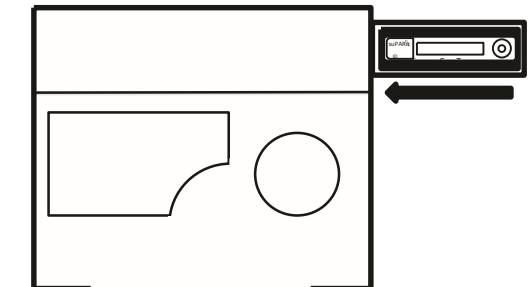
Procedure

1. Transfer 100 µl of Assay Running Buffer to an empty tube.
2. Transfer 10 µl of plasma sample to the tube containing 100 µl of Assay Running Buffer.
3. Set the pipette to 60 µl and mix by pipetting the content of the tube four times up and down in the pipette tip. Preferably use a vortexer.
4. Transfer 60 µl of diluted sample to the well of the suPARnostic® Quick Triage device.
5. Incubate for 20 min and insert the device into the reader before measurement. Press 'Start' to read the device.

DEVICE DESCRIPTION



suPARnostic® QUICK TEST READER INFORMATION

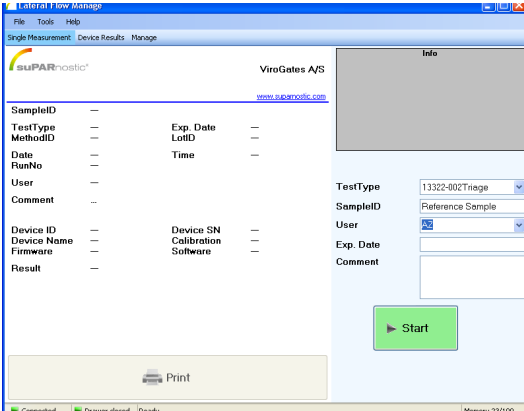


Insert device with patient ID to the left closest to the reader, and the sample well to the right. It is important to position the device correctly in the reader.

RUNNING A TEST

The LF Software allows the user to measure a device and get suPAR as a quantitative measure based on a calibration curve. Additionally, the user can select or enter a patient ID, user ID, expiry date information and comment.

1. Click on the tab 'Single Measurement'. The software appears as shown below:



2. Ensure that the reader is connected and the PC and software is running.
3. Remove the device from its pouch.
4. Add the sample to the device as described in the assay procedure.
5. Wait 20 min for incubation to finish.
6. Insert the device with patient ID to the left closest to the reader, and the sample well to the right, when the incubation is done. Press 'Start' to start measuring the device.
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.

When the Quick Triage device is used with the suPARnostic® Quick Test Reader it will automatically display if any error has occurred. The reader also has an internal quality control which is run every time the reader is turned on.

CALCULATION OF RESULTS

The suPARnostic® Quick Test Reader performs the calculation of the result. The suPARnostic® Quick Triage device has to be used with the reader to give correct values and the user may not evaluate the results by only visually inspecting the Quick Triage device.

The reader will 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 suPARnostic® Quick Test Reader needs a batch specific method for each batch of Quick Triage devices. The batch specific method is uploaded to the Reader when a new batch is used. The method contains a calibration curve that the reader uses to convert the T-Line's intensity to ng/ml suPAR.

The mathematical calculation is made with linear curve based on 6 Reference Samples with known concentrations and 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 diagnostic tests if available.

The substances listed below were tested for interference with the suPARnostic® Quick Triage. 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 - 23	

Rheumatoid factors: Samples from 16 patients with increased rheumatoid factor in the concentrations (0-1600 kIU/L) were analyzed. We observed no significant correlation to rheumatic factors (R2=0.33).

EXPECTED VALUES

All individuals have a measurable suPAR level, and in healthy blood donors (N=9305) the suPAR level is around 2 -3 ng/ml (25-27% range from 2,08 - 2,71 ng/ml)^[1], in patients attending emergency departments around 4-6 ng/ml^{[2],[3]} and in patients with severe disease and organ failure, suPAR is often in the double-digits^{[4],[5]}. The higher the suPAR level, the higher the risk of disease progression and the worse the prognosis.

PERFORMANCE CHARACTERISTICS

Limit of Blank (LOB)

Limit of blank show the variation of a blank sample (Buffer Only). Highest value from 3 batch validations.

Limit of Detection (LOD)

Limit of detection is the lowest possible detection of suPAR that is not a blank sample. Highest value from 3 batch validations.

Limit of Quantification (LOQ)

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 five 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%

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

X = suPARnostic ELISA Y = suPARnostic Quick Triage

REFERENCES

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

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5. Donadello K, Scolletta S, Taccone FS, Covajes C, Santonocito C, Cortes DO, et al: Soluble urokinase-type plasminogen activator receptor as a prognostic biomarker in critically ill patients. J Crit Care 2014, 29:144-149.

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REF			
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Do not reuse	Do not use if package is damaged or open.	Contains sufficient for <n> tests.	Use by