LA Screen

For the detection of lupus anticoagulant

SUMMARY

Lupus Anticoagulant (LA) is a (non-specific) acquired inhibitor of coagulation formed by a heterogeneous group of autoantibodies that interfere with phospholipid-dependent coagulation tests. Sensitive tests such as dilute Russell's Viper Venom Time (dRVVT) or LA-sensitive activated partial thromboplastin time (aPTT) are used for detection.
LA forms part of the laboratory criteria that allow the diagnosis of the antiphospholipid syndrome (APS). However, their presence can be detected in asymptomatic individuals or associated with other clinical situations.

PRINCIPLE

In LA Screen, Russell's viper venom initiates plasma coagulation by direct activation of Factor X to Factor Xa in the presence of calcium and a low concentration of phospholipids. In this way, LA Screen is more specific than aPTT since it eliminates the interaction with factors of previous stages, and is independent of anomalies in the contact phase and factors VIII and IX deficit. In the presence of Lupus Anticoagulant, clotting times are prolonged.
The tests mixing normal plasma pools, allow to exclude factor deficiencies that can also prolong clotting times. If they continue to be prolonged, the presence of Lupus Anticoagulant or other inhibitors is suspected, which must be confirmed by LA Confirm.

PROVIDED REAGENTS

Reagent A: mixture of Russell viper venom with calcium chloride and phospholipids. Lyophilized.

NON-PROVIDED REAGENTS

- Wiener lab.'s LA Confirm
- Wiener lab.'s LA Control
- Wiener lab.'s Coagulation Control N
- Pool of normal platelet-poor plasma

INSTRUCTIONS FOR USE

Reconstitute Reagent A with the volume of distilled water indicated in the vial. Mix well by inverting the vial to ensure complete resuspension of the lyophilized material. Incubate the reagent dissolved at room temperature for 30 minutes and gently rotate the vial before use.

WARNINGS

Reagents are for “in vitro” diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

The Reagent is stable in refrigerator (2-10ºC) until the expiration date shown on the box. Once reconstituted the reagent is stable for:

Reagent	37ºC	RT (< 25ºC)	2-10ºC	-20ºC
Reagent A	8 hours	24 hours	48 hours	1 month

SAMPLE

Citrated plasma
a) Collection: obtain blood carefully (avoiding stasis or trauma) and place in a tube with anticoagulant in 9+1 exact ratio (example: 4.5 ml blood + 0.5 ml anticoagulant). Mix gently and centrifuge for 15 minutes at 2500 g. To obtain platelet poor plasma, transfer the plasma to a clean plastic tube and centrifuge for an additional 15 minutes.
b) Additives: to obtain plasma use Anticoagulante TP from Wiener lab. or 130 mmol/l (3.8%) or 109 mmol/l (3.2%) sodium citrate.
c) Known interfering substances: samples containing clots and/or abnormal hematocrits should be discarded. The icteric, lipemic or hemolyzed samples should be analyzed by manual techniques as some photometric instruments yield false readings.
No interference was observed by 1 IU/ml heparin.
Refer to the literature of Young for effects of drugs on the present method.
d) Stability and storage instructions: the plasma must be kept at room temperature until the test is performed. This period should not exceed more than 4 hours. In case plasma is not processed within that period, it may be frozen up to 6 months at -20ºC.

REQUIRED MATERIAL (non-provided)

- Hemolysis tubes
- Pipettes and micropipettes for measuring the stated volumes
- Stopwatch
- Water bath at 37ºC

PROCEDURE

Preheat excess Reagent A at 37ºC, at 200 ul/assay.

In a hemolysis tube place:	
Sample or **Control**	200 ul
Incubate for 1 minute at 37ºC. Then add:	
Reagent A (at 37ºC)	200 ul

Simultaneously start stopwatch. Stir briefly to homogenize the content, keep in water bath for approximately 20 seconds. Then remove the tube from the bath, gently tilt once per second and stop the timer at the time of clot formation. Automatic or semiautomatic instruments may be used for reading the results, detecting the formation of fibrin clots by photo-optical or mechanical methods.
Note the clotting time.
Repeat to obtain duplicate values and record the average as a result.

CALCULATION OF RESULTS

If the clotting time with LA Screen is within the reference range further testing for the presence of LA is not required. If the time with LA Screen is above the normal range, the investigation must continue by performing a mixing test with normal plasma pool and a confirmatory test using LA Confirm. According to the ISTH (International Society of Thrombosis and Haemostasis) recommendations in its 2009 update, the results should be expressed as a normalized ratio, obtained after dividing the patient's result by the value corresponding to a normal pool

$$\text{LA Screen ratio} = \frac{\text{Patient´s LA Screen time (sec)}}{\text{Normal pool LA Screen time (sec)}}$$

If the value of the LA Screen Ratio is greater than 1.20, the presence of LA should be suspected.
If you have to use the LA Confirm confirmatory test, proceed in the same way to standardize it:

$$\text{LA Confirm ratio} = \frac{\text{Patient´s LA Confirm time (sec)}}{\text{Normal pool LA Confirm time (sec)}}$$

Then calculate the normalized LA Ratio of the patient, dividing LA Screen and LA Confirm normalized ratios:

$$\text{Normalized LA ratio} = \frac{\text{LA Screen ratio}}{\text{LA Confirm ratio}}$$

Example:
Patient´s LA Screen time: 42.1 sec
Normal LA Screen Average time: 37.5 sec
Patient´s LA Confirm time: 34.7 sec
Normal LA Confirm average time: 34.0 sec

$$\text{Normalized LA ratio} = \frac{(42.1 / 37.5)}{(34.7 / 34.0)} = \frac{1.12}{1.02} = 1.1$$

INTERPRETATION OF RESULTS

If the LA Screen Ratio is greater than 1.20, perform a test by mixing the patient´s plasma with a pool of normal platelet-poor plasma in a 1 + 1 ratio to identify a deficit of one or more factors or a treatment with Vitamin K antagonists. If no correction is evident with the pool of normal plasmas, it is possible to confirm the presence of an antiphospholipid nature inhibitor like LA.
In antiphospholipid syndrome, persistence of LA should be confirmed in a second sample drawn from the patient at least 12 weeks later.
For an adequate diagnostic procedure refer to the guidelines developed by the different scientific committees (International Society of Thrombosis and Hemostasis in its 2009 update, British Committee for Standards in Haematology published in 2012 and Clinical and Laboratory Standards Institute of 2014).

REFERENCE VALUES

For LA Screen: 31-44 seconds
For LA Confirm: 30-38 seconds
Normalized LA ratio = 0.8-1.2

Reference values vary between laboratories depending on the techniques and systems used. Therefore, each laboratory should establish its own reference intervals.

QUALITY CONTROL METHOD

Wiener lab.'s LA Control & Coagulation Control N

PROCEDURE LIMITATIONS

Erroneous results may be obtained for samples with heparin concentrations > 1 U / mL, in patients taking oral anticoagulants and in DIC.
For the results to be comparative, the LA Screen and LA Confirm tests must be performed simultaneously and in the same sample.
Complete removal of platelets from plasmas should be ensured by appropriate centrifugation, as phospholipids interfere with the tests.
Given LA heterogeneity, it is important to include two screening tests of different principles (dRVVT/aPTT).

PERFORMANCE

a) Reproducibility: determined with different samples (in series and daily). The following results were obtained:

Intra-assay precision

Level	S.D.	C.V.
62,4 sec	0.35 sec	0.56%
42,5 sec	0.41 sec	0.97%

Inter-assay precision

Level	S.D.	C.V.
62,4 sec	0.60 sec	0.96%
42,5 sec	0.87 sec	2.05%

b) Sensitivity: 12 positive results were found in 12 patients with clinical evidence of lupus anticoagulant.
c) Specificity: samples of normal patients treated with heparin and coumarin were tested, obtaining the following results:

Samples	Results > 1.2 (false positive)
Normal	1.2% (1/79)
Plasma with coumarin	0% (0/14)
Plasmas with heparin	5.5% (1/18)
Factors VIII and IX deficient plasmas	0% (0/6)

PARAMETERS FOR AUTOANALYZERS
For programming instructions consult the user manual of the analyzer in use.

WIENER LAB PROVIDES
5 x 2 ml (Cat. Nº 1705023)

REFERENCES

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- D. A. Triplett: Screening for the Lupus Anticoagulant. Research in Clin. and Lab. 19 (1989); 379.
- Pengo V, Tripodi A, Reber G, et al; Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientificand Standardisation Committee of the International Society on Throm-bosis and Haemostasis. Update of the guidelines for lupus antico-agulant detection. J Thromb Haemost 2009;7(10):1737–1740.
- Keeling D, Mackie I, Moore GW, Greer IA, Greaves M; British Committee for Standards in Haematology. Guidelines on the investigation and management of antiphospholipid syndrome. Br J Haematol 2012;157(1):47–58.
- Recent guidelines and recommendations for Laboratory Detection of Lupus Anticoagulants. Gary W. Moore, BSc, DBMS, CSci, FIBMS, CBiol, MSB, CerMHS. Semin. Throm. Hemost. 2014; 40:163-171.
- Guidelines on the Investigation and Management of antiphosplipid syndrome. David Keeling, Ian Mackie, Gary W. Moore, Ian A. Greer, Michael Greaves and British Committee for Standards in Haematology. British Journal of Haematology, 2012 157, 47-58.

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

Authorized representative in the European Community

"In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Contents

Batch code

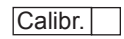
Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use



Calibrator

Control

Positive Control

Negative Control

Catalog number

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