LupoTek DetecTin VL
LupoTek CorrecTin VL

Lupus Anticoagulant Testing Kits

INTENDED USE

LupoTek DetecTin VL and **CorrecTin VL** test kits are qualitative tests intended to aid in the detection of lupus anticoagulants (LA) in citrated plasma by the dilute Russell's viper venom method in professional clinical laboratories.

SUMMARY

Lupus anticoagulants are antiphospholipid autoantibodies targeted against complexes of proteins and negatively charged phospholipids. Clinically they are associated with auto-immune disease (1), recurrent fetal loss (2) and unexplained thrombosis, both venous and arterial (3).

Circulating anticoagulants are usually detected by the presence of a prolonged clotting time in global coagulation tests (4) which does not correct on mixing patient plasma (1:1) with normal plasma. These tests are not specific, and cannot distinguish between a factor inhibitor, heparin contamination and a true antiphospholipid antibody without further studies.

The hallmark characteristic of lupus anticoagulants is their phospholipid dependence; that is, the prolonged clotting time seen with low phospholipid reagents is corrected with high phospholipid reagents.

The Dilute Russell Viper Venom time (DRVVT) is a simple one stage clotting test which can be used with carefully matched low and high phospholipid reagents to detect Lupus Anticoagulants with minimal interference from other types of circulating anticoagulants (5).

PRINCIPLE

LupoTek DetecTin VL and LupoTek CorrecTin VL use *Vipera lebetina* venom rather than *Vipera russelli* (Russell's Viper) venom. *Vipera lebetina* venom, like Russell's viper venom, will directly activate Factor X without requiring Factor VII. The activated Factor X in conjunction with Factors V, II, calcium ions and phospholipid will generate thrombin which converts fibrinogen to fibrin, producing a clot in the test system.

LupoTek DetecTin VL, the low phospholipid reagent, is designed as the screening reagent to detect a prolongation of the clotting time. **LupoTek CorrecTin VL** is the high phospholipid reagent that neutralizes the LA and corrects the clotting time to normal, confirming the presence of a Lupus Anticoagulant.

REAGENTS

WARNING: FOR *IN-VITRO* DIAGNOSTIC USE ONLY.

LupoTek DetecTin VL. Catalog number 85-202. 10 x 2 mL vials.

Ingredients: *Vipera lebetina* venom, low concentration of phospholipids, anti-heparin agents, calcium ions, buffers, stabilizers and a blue dye. Sodium azide (0.05%) is used as a preservative.

Preparation for Use: Reconstitute the vial with ***2 mL*** distilled water. Mix well, do not shake and leave at room temperature for 10 minutes before use.

LupoTek CorrecTin VL. Catalog number 86-201. 10 x 1 mL vials.

Ingredients: *Vipera lebetina* venom, high concentration of phospholipids, anti-heparin agents, calcium ions, buffers, stabilizers and a pink dye. Sodium azide (0.05%) as a preservative.

Preparation for Use: Reconstitute the vial with ***1 mL*** distilled water. Mix well, do not shake and leave at room temperature for 10 minutes before use.

Storage and Stability

The lyophilized reagents are stable until the expiration date printed on the vials. After reconstitution, the reagents are stable for 24 hours at 2-8^{0} C or 8 hours at room temperature.

WARNING: SODIUM AZIDE. Both **LupoTek DetecTin VL** and **LupoTek CorrecTin VL** contain sodium azide, which can form highly explosive metal azides if exposed to lead or copper in plumbing. Any such materials should be discarded into a sink with large volumes of water to minimize such a risk.

SPECIMEN HANDLING

Specimen Collection and Preparation

Plasma is obtained from whole blood anti-coagulated with 1 part 3.2% sodium citrate to 9 parts whole blood. Process the collected whole blood and handle the plasma according to the CLSI guideline H21-A5 (11).

To insure an optimum sample, the plasma should contain less than 10 x 10^{9}/L platelets (6). Double centrifugation or filtration through a 0.22 micron (μ) syringe type filter before testing is recommended (7). This is particularly important if plasma is to be frozen before testing.

Storage and Stability

Store plasma according to the CLSI guideline H21-A5 noted above. It is strongly recommended that plasma should be double centrifuged or filtered before freezing as outlined above. Any residual platelets will rupture on freezing and thawing and can neutralize a lupus anticoagulant by exposure of phospholipids from the damaged membranes. Thaw rapidly at 37°C before use.

Materials Required but not Provided

Pooled Normal Plasma

TEST PROCEDURE

Please contact r2 Diagnostics for validated applications for LupoTek Detectin VL and LupoTek Correctin VL on individual models of coagulation analyzers.

Quality Control

Quality control of coagulation tests involves multiple components. Each laboratory should establish a quality control program that includes both normal and abnormal control plasmas. r2 Diagnostics' normal control plasma PlasmaCon N and LA positive control plasma PlasmaCon LA are suitable controls. Quality control testing with the **LupoTek DetecTin VL** and **CorrecTin VL** reagents should be carried out at the same time.

RESULTS

For optimum results, testing using **LupoTek DetecTin VL** and **CorrecTin VL** should be done at the same time. If the **LupoTek DetecTin VL** time is in the normal range no further testing is necessary. If the clotting time with the **DetecTin VL** reagent is above the upper limit of the normal reference range further testing with the **CorrecTin VL** reagent is justified.

The clotting times obtained with the **DetecTin VL** and **CorrecTin VL** reagents are used to express results in a ratio format, as outlined below. The use of a **normalized ratio (NR)**, in which the patient's clotting time is divided by the clotting time of pooled normal plasma, minimizes any impact of differences in the normal ranges due to lot to lot reagent variability:

$$\mathbf{NR} = \frac{\dfrac{\text{DetecTin VL Time of Patient}}{\text{DetecTin VL Time of Pooled Normal Plasma}}}{\dfrac{\text{CorrecTin VL Time of Patient}}{\text{CorrecTin VL Time of Pooled Normal Plasma}}}$$

INTERPRETATION OF RESULTS

In an internal study of 122 patient samples a ROC analysis suggested a cutoff of 1.30 for the Normalized Ratio. Samples above this level were considered as LA positive.

The example cutoff value described above is not absolute and each laboratory is encouraged to establish its own specific ratios for its normal reference and patient populations.

Patient plasmas yielding borderline ratios should be repeated and correlated with the clinical findings if indicated. Patient plasmas that have long clotting times with both **LupoTek DetecTin VL** and **CorrecTin VL**, irrespective of the ratio, may have some other defect such as a Factor deficiency or be on oral anticoagulant therapy.

Mixing Studies

Mixing studies are recommended to differentiate between factor deficiency states and circulating inhibitors (6). A failure to correct on mixing with normal plasma (1:1) is more indicative of an inhibitor, while correction is more suggestive of a factor deficiency state. Mixing studies should be carried out under carefully controlled conditions using well characterized pooled normal plasma (8, 9).

REFERENCE VALUES

Reference ranges for normal plasmas with **LupoTek DetecTin VL** are in the **34-54** second range when assessed on the STA Compact.

Reference ranges for normal plasmas with **LupoTek CorrecTin VL** are in the **31-44** second range when assessed on the STA Compact.

These results are illustrative only. Each laboratory must establish its own normal references ranges for the technique and/or instrument used in the laboratory.

LIMITATIONS

All testing for lupus anticoagulants require plasma samples that are platelet poor (<10 x 10^9/L) or preferably platelet free. Samples not double centrifuged or filtered should be tested before freezing. Both DetecTin VL and CorrecTin VL tests should be performed on either a fresh sample or a frozen sample.

Factor deficiencies, oral anticoagulants, and other antibody-type inhibitors can lengthen the clotting times of both LupoTek DetecTin VL and CorrecTin VL. Mixing studies are recommended to differentiate between the clinical conditions (6, 8, 9).

DetecTin VL and **CorrecTin VL** incorporate a heparin neutralization agent that is effective to 0.6 U/mL of unfractionated heparin. Testing of samples with higher heparin levels is not recommended. The effects of low molecular weight heparins and direct thrombin inhibitors have not been determined.

The performance characteristics of **DetecTin VL** and **CorrecTin VL** have not been evaluated in pediatric populations.

PERFORMANCE CHARACTERISTICS

Precision

CLSI EP5-A2 (10) precision estimates of LupoTek Detectin VL and Correctin VL on the STA Compact, as %CV of the clotting times, were:

Reagent	Plasma	Repeatability Imprecision	Total (Within Instrument) Imprecision
Detectin VL	Normal	1.3%	3.6%
	Abnormal	1.4%	3.7%
Correctin VL	Normal	1.1%	3.7%
	Abnormal	1.3%	2.6%

Interferences

Interference studies of LupoTek Detectin VL and Correctin VL were determined on a Stago STA Compact analyzer. Interferant was spiked into pooled normal plasma and the maximum concentration tolerated in the assay was defined as the highest concentration of interferant wherein any consistent shift relative to the recovered value of the base PNP clotting time was less than 10%. The maximum concentrations were:

Interferant class	Maximum Concentration Tested	Maximum tolerated concentration
Hemolysis	500 mg/dL hemoglobin	500 mg/dL hemoglobin
Icterus	20 mg/dL unconjugated bilirubin	Icteric samples are not appropriate.
Lipemia	2,000 mg/dL triglycerides	2,000 mg/dL triglycerides
Heparin	2.0 U/mL unfractionated heparin	0.6 U/ml unfractionated heparin

Method Comparison

One hundred twenty-two patient samples were analyzed on the STA Compact in an internal study to determine the cutoff for the LupoTek Detectin VL / Correctin VL normalized ratio. Thereafter a total of another one-hundred fifty-five patient samples were analyzed in three laboratories using LupoTek Detectin VL and Correctin VL, and Diagnostica Stago DRVV Screen and Confirm reagents, on STA Compact analyzers. All samples were a mix of known LA patients and other miscellaneous clinical conditions.

Positive percent agreement and negative percent agreement were calculated using the normalized ratio of the test and predicate devices according the FDA guidance document 1620, "Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests". The results were:

Detectin VL/Correctin VL compared to Stago DRVV Screen/Confirm	
Statistic	Result
Positive Percent Agreement	98%
Negative Percent Agreement	96%

References:

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3) Ames PRJ. et al. Antiphospholipid antibodies, hemostatic variables and thrombosis-A survey of 144 patients. Thromb. Haemost: 1995; 73: 768-773.
4) Triplett DA., et al., Laboratory identification of the lupus anticoagulant. Br. J. Haematol: 1991; 73,: 139-142.
5) Exner T. et al. Use of a simplified dilute Russell's viper venom time (DRVVT) confirms heterogeneity among "lupus anticoagulants". Blood Coag. Fibrinol: 1990; 1, 259-266.
6) Brandt JT. et al. Criteria for the diagnosis of Lupus Anticoagulants: An update. Thromb. Haemost: 1995;74(4), 1185-1190.
7) Sletnes KE. et al. Preparation of plasmas for the detection of lupus anticoagulants and antiphospholipid antibodies. Thromb. Res: 1992; 65. 43-53.
8) Marques MB and Fritsma GA, Quick Guide to Coagulation Testing, AACC Press2006.
9) Laposata M, et. al., The Clinical Hemostasis Handbook, Year Book Medical Publishers, 1989.
10) EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-2nd Edition, Clinical Laboratory Standards Institute, 2004.
11) H21-A5, Collection, Transport and Processing of Blood Samples for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition, Clinical Laboratory Standards Institute, 2008.

R2 Diagnostics, Inc.
South Bend, Indiana USA (574) 288-4377

EC REP Enzyme Research Laboratories, Ltd.
Swansea, UK