



ISSN (P): 2617-7226  
ISSN (E): 2617-7234  
[www.patholjournal.com](http://www.patholjournal.com)  
2020; 3(4): 12-15  
Received: 27-08-2020  
Accepted: 09-10-2020

**Dr. Pramod Kumar Pamu**  
Assistant Professor,  
Department of Pathology,  
Nizam's Institute of Medical  
Sciences, Punjagutta,  
Hyderabad, Telangana, India

## Lupus anti-coagulant (LA) screening & confirmation testing by dilute Russell viper venom time (DRVVT) method

**Dr. Pramod Kumar Pamu**

DOI: <https://doi.org/10.33545/pathol.2020.v3.i4a.305>

### Abstract

**Background:** Anti-phospholipid antibody syndrome is a life-threatening acquired disorder of the immune system which can lead to dysfunction of the multiple systems in the human body. Lupus anticoagulant (LA) detection by the normalized dilute Russell viper venom time (DRVVT) ratio provides a robust assay methodology with accurate results.

The objective of this study was to evaluate the potential ability of diluted Russell viper-venom time (DRVVT) to identify Lupus anticoagulant (LAC) by screening and confirmation testing methods.

**Methods:** Samples of the suspected auto-immune disorder patients with lengthened screening DRVVT (using LA Check) more than 2 standard deviations (SD) above the average level of normal donors undergo additional testing: a 1:1 blend with normal plasma which taken from various normal individuals and amalgamated together (using LA Check) and high phospholipid confirmatory testing (using LA Sure).

**Results:** A total of 311 cases included in this study. Out of 311, 230 patients were females and 81 were males. Out of 230 females LAC positivity by DRVVT method was seen in 78 patients and 152 were Negative. Out of 81 males LAC positivity by DRVVT method was seen in 38 patients and 43 were Negative.

**Conclusions:** Precise evaluation of lupus anticoagulant plays a major role in estimating the risk factors for thrombosis. A practically elevated lupus anticoagulant result may change the duration of anticoagulation medications usage for patients. The DRVVT is recognized and certified by all three expert panels as one of the preliminary assays to be included when screening for a LAC.

**Keywords:** Dilute russell viper venom time, lupus anti-coagulant, thrombosis, phospholipids

### Introduction

In 1972, hematologist C. Lockard Conley coined the term lupus anticoagulant <sup>[1]</sup>. All the patients who get a positive test result for lupus anticoagulant do not have systemic lupus erythematosus/SLE, bleeding manifestations occur only when there is contemporaneous thrombocytopenia or factor deficiency or factor inhibitor. Only presence of lupus anticoagulant is not an absolute risk factor for bleeding. Antiphospholipid antibodies are a single of group of antibodies which are constantly circulating in the blood and bind to the proteins present in the plasma and ultimately bind to phospholipids singly or in group and thus lead to pathogenesis and development of the disease of abnormality in the coagulation cascade. Anti-phospholipid antibody syndrome is an acquired life threatening autoimmune disorder which can lead to dysfunction of the multiple systems in the human body which can range from recurrent thrombosis in veins and arteries in various parts of the body, can lead to intrauterine death or recurrent pregnancy losses and other obstetric complications <sup>[2, 3]</sup>. Primary Anti-phospholipid syndrome develops in the absence of any pre-existing disease and secondary Anti-phospholipid syndrome occurs in conjunction with other medical disease which is already present. Diagnosis of Anti-phospholipid syndrome requires presence of atleast one clinical and laboratory test abnormality. Lupus Anti-coagulant (LA/LAC), Anti-Cardiolipin Antibody (aCL/ACA) and Anti- Beta-2-Glycoprotein 1 are the group of acquired anti-phospholipid antibodies. Laboratory diagnosis of lupus anticoagulants has become very common in clinical practice due to a relative high occurrence rate and their persistence in the body is associated with several arterial and venous thrombotic events.

**Corresponding Author:**  
**Dr. Pramod Kumar Pamu**  
Assistant Professor,  
Department of Pathology,  
Nizam's Institute of Medical  
Sciences, Punjagutta,  
Hyderabad, Telangana, India

Accurate detection of lupus anti-coagulants is little tough because no single assay is total sensitivity or total specificity, thus laboratory methodologies typically do the test with two clot-based low-phospholipid (PL) screening methods designed to be sensitive to LA [4, 5]. The activated plasma thromboplastic time (aPTT) and dilute Russell viper venom time (DRVVT) amalgamation is the most extensively used to get good results and also to fulfill the criteria of International Society on Thrombosis and Haemostasis (ISTH) Scientific Standardization Committee guidelines [4].

**Aim of the Study**

The objective of this study was to evaluate the potential ability of diluted Russell viper-venom time (DRVVT) to identify Lupus anticoagulant (LAC) by screening and confirmation testing methods.

**Methods**

This is a retrospective record bases study done at a tertiary care center. Study period June 2016 to May 2018. Total number of patients included in this study was 311. The diagnostic efficiency and utility of any test is dependent upon the selection of the patient with his/her disease condition, clinical timing of the tests like morning or evening, alternatives available of the same type of tests, absolute and relative indications of the test, the various factors which can cause hindrance to the sample or the test, standards used for the tests and the interpretation of the test results. Therefore LAC also has all these limitations during the tests and results which are dependent on various factors as mentioned above. In the following sections we will discuss each of the above factors. Patient selection was done using Pengo *et al* criteria [4]:- 1) patients with unjustifiable venous thromboembolism, 2) inscrutable thrombosis in the arteries of the younger patients especially below 50 years of age, 3) Thrombosis at ectopic or not so common sites, 4) pregnancy loss in the middle aged and recurrence of pregnancy loss, 4) Any events of thrombosis or pregnancy complications in patients with co-existence of other autoimmune diseases like systemic lupus erythematosus (SLE), 5) In other patients though not most appropriate but still in whom testing is reasonable include individuals, 6) In patients in whom testing for antiphospholipid antibodies does not yield good results or not at all indication, 8) Old patients with recurrent venous or arterial thromboembolism, 9) and in group of individuals who are totally asymptomatic but tests show unexplained prolonged a PTT in whom work up for APLA is occasionally useful.

**Test Timing**

To get accurate results, testing for Lupus-Anticoagulant should be carried out when a patient is not having an acute episode of thrombosis and not receiving any anticoagulants medications which can derange the test values. We have followed this process during the testing and study period.

We have taken platelet free plasma for the testing of LAC as platelets interfere with the coagulation and leads to shorter coagulation time.

Firstly, screening tests such as APTT and DVRRT were done. The Activated Partial Thromboplastin Time (APTT) test assay is often used as a screening test to assess the comprehensive coherence of the intrinsic/common pathway of the coagulation cascade system. In this study, dilute Russell viper venom time (DRVVT) testing was performed with the Precision Biologic (Dartmouth, NS, Canada) LA Check and LA Sure manufacturer reagents. Samples with prolonged screening dRVVT (using LA Check) higher than 2 standard deviations (SD) higher than the average of normal donors undergo additional testing: a 1:1 blend with pooled normal plasma (using LA Check) and high phospholipid confirmatory testing (using LA Sure). The Staclot® LA 20 was performed (Diagnostica Stago, Parsippany, NJ), estimating or calculating a delta between the clotting times using the low phospholipid (screen) and high phospholipid (confirm) reagents. In this study, delta positivity cut-off was taken as per the manufacturer’s instruction which is the normal population mean + 4 SD. During the study period, the cut-off for positivity was a Δ of 8 seconds. The aPTT and LA assays were performed on a STA-R Evolution analyzer (Diagnostica Stago), which uses electro-mechanical clot detection.

$$SR = \frac{\text{Screen clotting time of test plasma}}{\text{Screen clotting time of reference pool}}$$

$$CR = \frac{\text{Confirm clotting time of test plasma}}{\text{Confirm clotting of time of the reference pool}}$$

$$\text{Normalized ratio (NR)} = \frac{SR}{CR}$$

The NR of ≥ 1.20 was taken as positive for LA as per manufacturer guidelines SR=Screen ratio; CR=Confirm ratio.

**Results**

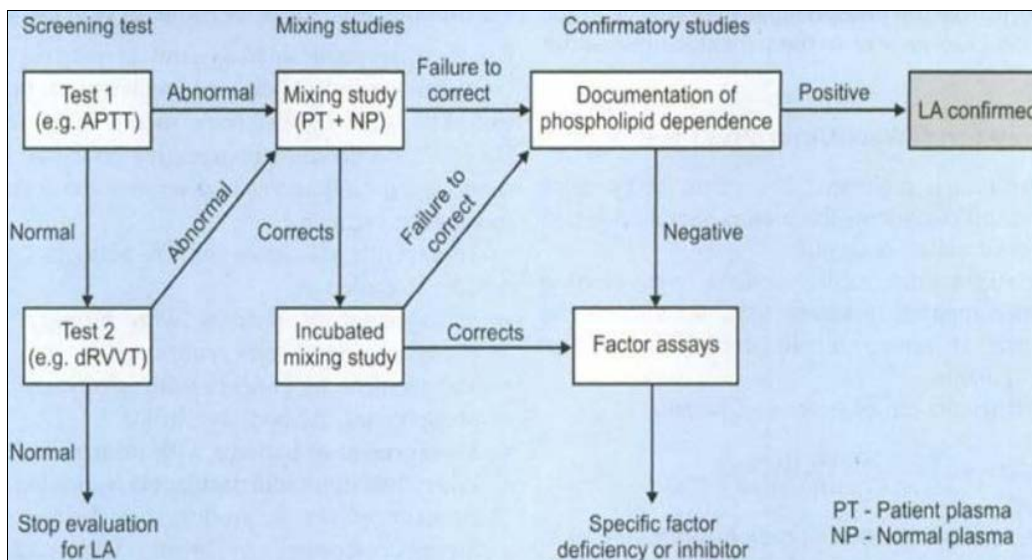
Total requisitions received for Lupus Anti-Coagulant (LAC) test were 584. 273 cases were excluded as they did not meet the criteria, a total of 311 cases were included in the study. Patients between ages 3 and 58 were included in this study. Male to Female ratio was 1:5 and the average age of showing up of symptoms was 28.8 years. Out of 311, 230 patients were females and 81 were males. Out of the 230 females most common clinical disease present in them was systemic lupus erythematosus -110 cases (48%) followed by recurrent pregnancy loss, DVT and others clinical diseases. Out of 230 females LAC positivity by DRVVT method was seen in 78 patients and 152 were Negative (Table 1). Out of 81 males LAC positivity by DRVVT method was seen in 38 patients and 43 were Negative (Table 2).

**Table 1:** Showing Lupus Anti-Coagulant Reactivity in Females

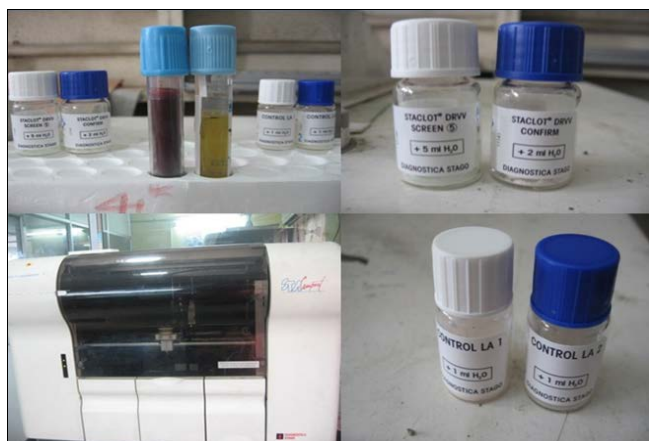
| Clinical Disease                        | Total No. of Cases (%) | Positive for LAC NR => 1.2 | Negative for LAC NR=<1.2 |
|---|------------------------|----------------------------|--------------------------|
| Systemic Lupus Erythematosus (Lupus)    | 110 (48 %)             | 44 (56.5%)                 | 64 (42.0 %)              |
| Recurrent Pregnancy Loss                | 59 (25.6 %)            | 21 (27 %)                  | 36 (23.6 %)              |
| Deep Vein Thrombosis (DVT)              | 27 (11.6%)             | 06 (7.7 %)                 | 21 (13.9 %)              |
| Cerebro-Vascular Accidents (CVA-Stroke) | 19 (8.3 %)             | 03 (3.7 %)                 | 16 (10.6 %)              |
| Other diseases                          | 15 (6.5 %)             | 04 (5.0 %)                 | 15 (9.9 %)               |
| Total                                   | 230                    | 78                         | 152                      |

**Table 2:** Showing Lupus Anti-Coagulant Reactivity in Females

| Clinical Disease                        | Total No. of Cases (%) | Positive for LAC NR => 1.2 | Negative for LAC NR < 1.2 |
|---|------------------------|----------------------------|---------------------------|
| Deep Vein Thrombosis (DVT)              | 34(42.0 %)             | 19 (50.0%)                 | 12 (27.9 %)               |
| Cerebro-Vascular Accidents (CVA-Stroke) | 16 (19.7%)             | 10 (26.3%)                 | 08 (18.6 %)               |
| Congenital Heart Disease                | 23 (28.3%)             | 03 (7.9%)                  | 17 (39.6 %)               |
| Post Renal Transplant                   | 06 (7.40 %)            | 05 (13.2%)                 | 02 (4.70 %)               |
| Others                                  | 02 (2.51 %)            | 01 (2.60%)                 | 04 (9.33 %)               |
| <b>Total</b>                            | 81                     | 38                         | 43                        |



**Fig 1:** Work-up Chart



**Fig 2:** Photograph showing Patient Blood Sample, Reagents used for Test and the Equipment Used for the Test

**Discussion**

Diagnosing and confirmation of the LAC in patients with relevant history helps the clinician in establishing the duration of the anti-coagulant therapy and also helps in preventing the recurrence of clinical features and morbidities [6, 7]. This is a retrospective study involving 311 patients and the diagnosis of LAC was made by using DRVVT, detection rate in females was 80.9 % and in males was 46.91%.

The clinical examination and laboratory tests play a crucial role in the diagnosis of a LAC. However, there are minor differences in laboratory practices in terms of the choice of assays for LAC, which depends on practices and outcomes [8]. For the purpose of improving diagnostic accuracy of LAC testing, top and expert panelists from various countries have put forth guidelines for laboratories to follow that

cover the pre-analysis phase, analytical phase, and post analysis phases of LAC testing procedure.

The guidelines for the test and interpretation have been proposed by: 1) the International Society of Thrombosis and Haemostasis Scientific Standardization Committee (ISTH SSC), which published revised guidelines in 2009; 2) the British Committee for Standards in Hematology (BCSH), which published guidelines in 2012; and 3) the Clinical and Laboratory Standards Institute (CLSI), which published guidelines in 2014 [9, 10]. There are various LAC assays which interact with the coagulation cascade and few of them, which are more commonly used are dilute Russell Viper Venom Test (dRVVT), Silica Clotting Time (SCT), Hexagonal Phase Phospholipid Neutralization (STACLOT-LA), Kaolin Clotting time (KCT) and dilute Prothrombin Time (dPT). ISTH SSC panel recommended laboratories to perform atleast two different assays which have two different principles because a single assay cannot detect LAC due to the heterogeneity.

First suspicion for the existence of a LAC usually starts with an unexplained elevated Activate Plasma Thromboplastin time (APTT). When APTT is raised, a mixing test to be done; 1:1 mixing of patient plasma with normal plasma. If the clotting of the 1:1 mix gets corrected, it implies a clotting factor deficiency. If it does not get corrected, it implies a circulating inhibitor interfering with the clotting mechanism. If there is no correction in the 1:1 mixing study and no reported history of bleeding in the patient, clinicians usually request a work-up for a LAC. This work-up can involve a single assay or a panel of assays.

The dRVVT is recognized and certified by all three expert panels as one of the preliminary assays to be included when screening for LAC. It has been shown by a various

investigators to be sensitive to B2GPI-dependent antibodies and to correlate very strongly with thrombosis<sup>[11]</sup>.

The dRVVT test is a dual component test. The initial component is a screening reagent which contains a reduced sum of phospholipids in the reagent, which make the reagent very sensitive to the presence of a LAC. When patient plasma or the test plasma is having LAC is added on to the screening reagent, the clotting time is extended or prolonged because the antibodies present in the plasma interfere with the prothrombinase complex's ability to bind to the phospholipid surface. The second component is a confirmatory reagent, which has a higher amounts of phospholipids added to the reagent. The additional phospholipids neutralize the antibodies by providing an wide surface area for attachment and unalterable binding of the prothrombinase complex. This leads to a shortening of the clotting time compared to the screening assay. When the screen and confirm are used together, they can be considered an "integrated" assay<sup>[12]</sup>.

This study is in correlation with the study conducted by Jacquot *et al.* where the positivity (prolonged screening time) was seen in more than 60% of the cases and this study showed more than 80 % positivity.<sup>[13]</sup> Conversely, we show that patients with positive dRVVT by both ISTH and manufacturer guidelines are more likely to have positive immunoassays.

### Conclusions

Correct and timely detection of lupus anticoagulant (LAC) is utmost significance as it is a major risk factor for thrombosis of arteries and veins. Due to the heterogeneity of lupus anticoagulant (LAC) leads to the inability to create a single gold standard diagnostic assay; and because of this several different assays are used to detect it so as to get the accurate results. The interpreter should be aware of the test results, interferences and limitations of the test assay. All the laboratories should apply the DRVVT screening and confirmation as a primary means for LAC identification and confirmation which should be in confirmation specified in ISTH guidelines.

### References

1. Feinstein DI, Rapaport SI. Acquired inhibitors of blood coagulation. *Prog Hemost Thromb* 1972;1:75-95.
2. Lubbe WF, Liggins GC. Lupus anticoagulant and pregnancy. *Am J Obstet Gynecol* 1985;153(3):322-327. doi:10.1016/s0002-9378(85)80124-1
3. Branch DW, Scott JR, Kochenour NK, Hershgold E. Obstetric complications associated with the lupus anticoagulant. *N Engl J Med* 1985;313(21):1322-1326.
4. Pengo V, Tripodi A, Reber G, J H Rand, T L Ortel, M Galli *et al.* Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost* 2009;7(10):1737-1740.
5. Favaloro EJ, Wong RC. Laboratory testing for the antiphospholipid syndrome: making sense of antiphospholipid antibody assays. *Clin Chem Lab Med* 2011;49(3):447-461.
6. Greaves M, Cohen H, MacHin SJ, Mackie I. Guidelines on the investigation and management of the

- antiphospholipid syndrome. *Br J Haematol* 2000;109(4):704-715.
7. Hirsh J, Dalen JE, Anderson DR, Poller L, Bussey H, Ansell J *et al.* Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. *Chest* 2001;119(1):8S-21S.
8. Dembitzer FR, Ledford Kraemer MR, Meijer P, Peerschke EI. Lupus anticoagulant testing: performance and practices by North American clinical laboratories. *Am J Clin Pathol* 2010;134(5):764-773.
9. 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.
10. Clinical, Laboratory Standards Institute (CLSI). Laboratory Testing for the Lupus Anticoagulant; Approved Guideline. CLSI document H60-A. Wayne, PA, Clinical Laboratory Standards Institute, 2014.
11. Tripodi A. Laboratory testing for lupus anticoagulants: a review of issues affecting results. *Clin Chem* 2007;53(9):1629-1635.
12. Teruya J, West AG, Suell MN. Lupus anticoagulant assays: questions answered and to be answered. *Arch Pathol Lab Med* 2007;131(6):885-889.
13. Jacquot C, Wool GD, Kogan SC. Dilute Russell viper venom time interpretation and clinical correlation: A two-year retrospective institutional review. *Int J Lab Hematol* 2019;41:80-86.