

## **SUPPLEMENTAL TECHNICAL BULLETIN ST – 2011 – 01**

### **Title: ISTH SSC 2009 Updated Guidelines for Lupus Anticoagulant Detection**

Pengo V, Tripodi A, Reber G, Rand JH, Ortel TL, Galli M, de Groot PG. Update of the guidelines for lupus anticoagulant detection. *J Thromb Haemost* 2009; 7: 1737–40.

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#### **Summary:**

One of the conclusions of the ISTH Scientific Subcommittee meeting on Lupus Anticoagulant/Phospholipid dependent antibodies, held in Geneva on 2007, was the need to update the guidelines on Lupus Anticoagulant (LA) detection. Particular emphasis was given to several aspects discussed in this official communication. A new paragraph is dedicated to the patient selection, and aims to minimize inappropriate requests for LA testing. Modalities for blood collection and processing are fully delineated and the choice of tests is limited to dRVVT and a sensitive aPTT. Calculation of cut-off values for each diagnostic step are clearly stated. A final paragraph reports the interpretation of the results in general and in particular situations. These guidelines are intended to update the criteria for the detection of the presence of lupus anticoagulants (LA) that were originally proposed by Brandt et al. in 1995 [1]. The Subcommittee on Lupus Anticoagulant/Phospholipid-dependent Antibodies acknowledges that the present guidelines have been extremely useful during the past 13 years but that it is now appropriate to provide additional details and specifications in light of the knowledge and experience that has been accumulated since their publication.

#### **Recommendations for the optimal laboratory detection of lupus anticoagulant (LA)**

##### **(A) Blood collection**

1. Blood collection before the start of any anticoagulant drug or a sufficient period after its discontinuation
2. Fresh venous blood in 0.109 M sodium citrate 9:1 ratio
3. Double centrifugation to prepare Platelet Poor Plasma (PPP, <10,000/ $\mu$ L)
4. Quickly frozen plasma is required if LA detection is postponed
5. Frozen plasma must be thawed at 37°C

##### **(B) Choice of the test**

1. Two tests based on different principles

2. dRVVT should be the first test considered
  3. The second test should be a sensitive aPTT (low phospholipid and only silica as activator)
  4. LA should be considered as positive if one of the two tests gives a positive result
  5. For interpretation see (screening test)
- (C) Mixing test
1. Pooled Normal Plasma (PNP) for mixing studies may be prepared in house. Adequate commercial lyophilized or frozen Pooled Normal Plasma (PNP) can alternatively be used
  2. A 1:1 proportion of patient: PNP shall be used, without preincubation within 30 min.
  3. LA can not be conclusively determined if the Thrombin Time (TT) of the test plasma is significantly prolonged
  4. For interpretation see (mixing test)
- (D) Confirmatory test
1. Confirmatory test(s) must be performed by increasing the concentration of Phospholipid (PL) of the screening test(s)
  2. Bilayer or hexagonal (II) phase PL should be used to increase the concentration of PL.
  3. For interpretation see Table 2 (confirmatory test)
- (E) Expression of results
1. Results should be expressed as ratio of patient-to-PNP for all procedures (screening, mixing and confirm)
- (F) Transmission of results
1. A report with an explanation of the results should be given

## **Cut-off values for lupus anticoagulant (LA) detection**

Screening test - How should this be determined

1. Perform testing on plasmas from healthy donors
2. Take the cut-off as the value above the 99th percentile of the distribution

Interpretation

1. Results of screening tests are potentially suggestive of LA when their clotting times are longer than the local cut-off value

Mixing test - How should this be determined

1. Perform testing on plasmas from healthy donors mixed with the pooled normal plasma (PNP) at 1:1 proportion. Testing should be performed without pre-incubation within 30 min
2. Take the cut-off as the value above the 99th percentile of the distribution
3. Alternatively, the cut-off may be the value of the ICA defined according to the equation:  
$$\text{ICA} = [(b - c)/a] \times 100$$
where a, b and c are the clotting times of the patient plasma, mixture and normal plasma, respectively [16]

Interpretation

1. Results of mixing tests are suggestive of LA when their clotting times are longer than the local cut-off value, or when the ICA is greater than the local cut-off value

Confirmatory test - How should this be determined

1. Perform testing on plasmas from healthy donors at low (screen) and high (confirm) phospholipid concentration
2. Take the cut-off as the value corresponding to the mean of the individual % corrections calculated as defined by the equation  $[(\text{screen} - \text{confirm})/\text{screen}] \times 100$

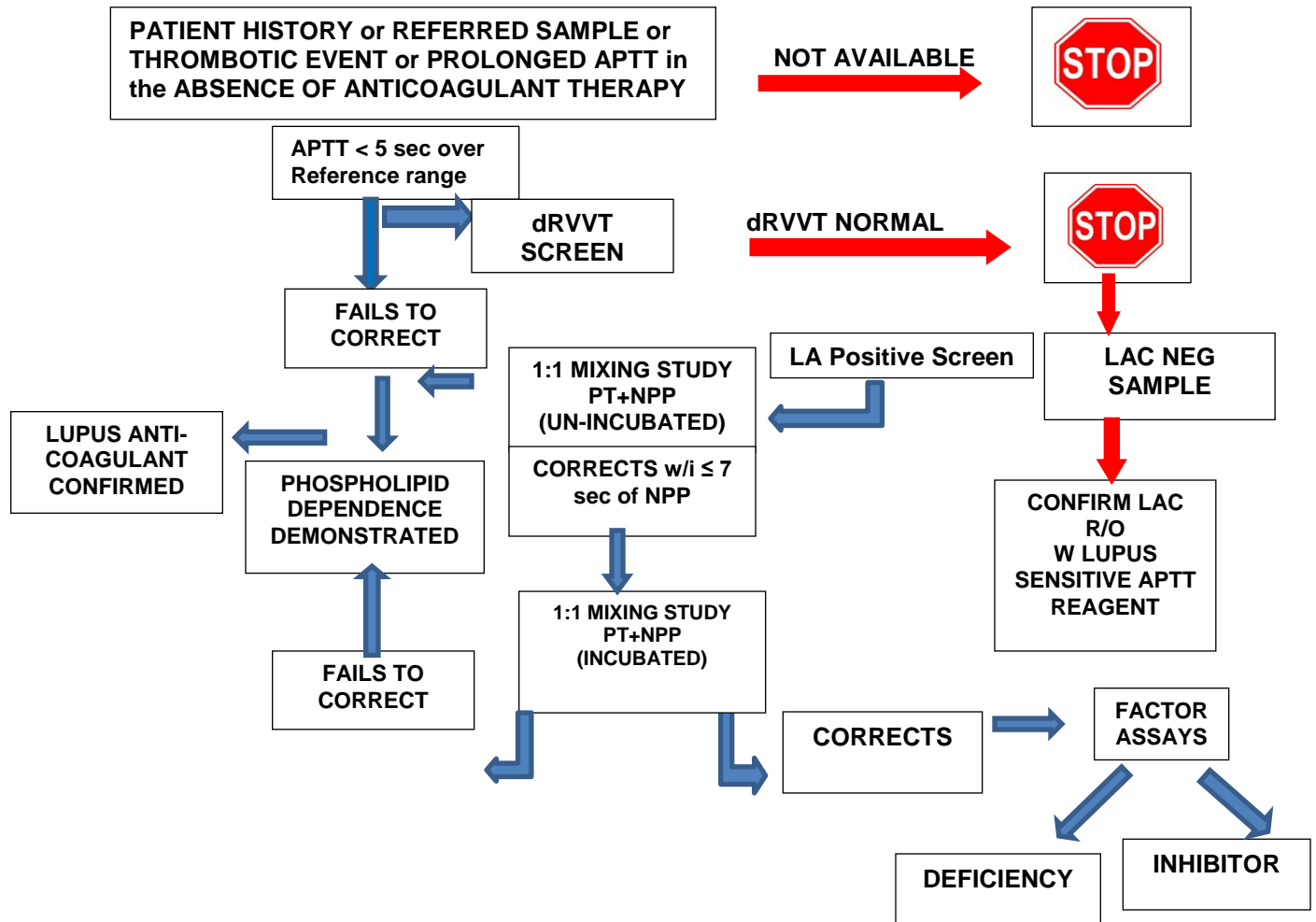
Interpretation

1. Results are confirmatory of LA if the % correction is above the local cut-off value

\*Testing described above must be performed with the local reagent/coagulometer combination on plasmas from at least 40 adult healthy donors less than 50 years of age. Do not use cut-off values established elsewhere even although they refer to the same method and coagulometer.

\*The clotting time of the confirmatory test in LA positive samples is not always shortened to within the normal range of controls. To avoid false-negative results, the Subcommittee recommends confirmatory tests to be performed in all the normal controls and to use the mean of obtained clotting times to calculate the percentage of shortening. This percentage can be used as a cut-off value. ICA, Index of Circulating Anticoagulant.

## LUPUS ANTICOAGULANT FLOWCHART



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