New Testing Algorithm: Lupus Anticoagulant Cascade

The Thrombosis and Hemostasis Laboratory is pleased to offer a Lupus Anticoagulant Cascade to aid in the diagnosis of anti-phospholipid antibody syndrome associated with thrombosis. This assay went live Wednesday June 15, 2016, and the Silica Clotting Time and Dilute Viper Venom will no longer be orderable as stand alone tests.

Anti-phospholipid syndrome (APS), the most common cause of acquired thrombophilia, is associated with significant morbidity and mortality across diverse patient populations. The most frequently detected antibodies are commonly referred to as lupus anticoagulants (LA) due to their prevalence in patients with systemic lupus erythematosus. However, the antibodies, known as anti-phospholipid antibodies (APA) associated with APS are extremely heterogeneous and are directed against a wide variety of anionic phospholipids, including cardiolipin, β2 glycoprotein 1 (B2GP1), cell-membrane phosphatidylerine, and many others. While these antibodies most commonly cause in vivo thrombosis, these same antibodies paradoxically prolong in vitro clot-based laboratory assays.

A panel of tests is necessary to detect APAs as no single test presently available is sufficient to detect (or exclude) this diverse group of antibodies. The LA Cascade is provided (see below) as an overview of the recommended laboratory testing and should not supplant the diagnostic interpretation provided by the Thrombosis and Hemostasis Laboratory.

LUPUS ANTICOAGULANT (LA) TESTING

Based upon consensus criteria from the International Society for Thrombosis and Haemostasis (ISTH), confirmation of a LA requires that the following criteria are met:

- Performing 2 or more phospholipid-dependent clotting tests demonstrating prolongation of at least one test (i.e. Silica Clot Time (SCT), dilute Russell Viper Venom Test (dRVVT))
- Evidence for inhibitory activity shown by the effect of patient plasma on normal pooled plasma. (i.e. mixing study which fails to show complete correction)
- Demonstration of phospholipid-dependence of the inhibitor on a confirmatory test as evidenced by shortening of the clotting time with the addition of additional phospholipid.

Equally important, the ISTH recommends the following are performed:

- Routine clotting tests such as the prothrombin time (PT) and partial thromboplastin time (aPTT) to evaluate for the possibility of other coagulation disorders, particularly those which interfere with LA testing methods
- Factor assays whenever there is a suspicion of a specific factor deficiency or inhibitor

The laboratory criteria include positive testing for one of the following on 2 or more occasions, at least 12 weeks apart:

1. Lupus anticoagulant
2. Cardiolipin antibodies (IgG or IgM) in medium or high titer, and/or
3. b2-glycoprotein 1 antibodies (IgG or IgM)
Though rare, a factor-specific antibody to factor VIII can result in false positive LA testing; as part of the diagnostic interpretation, the laboratory will ask the ordering medical provider to exclude the likelihood of a factor specific inhibitor. Factor activity assays can be performed upon request.

INTERPRETATION OF LABORATORY TEST RESULTS

The Clinical Laboratory Standards Institute (CLSI) published updated 2014 guidelines for the laboratory diagnosis of APA. These guidelines state that all laboratory results and calculations in the laboratory’s LA panel must undergo a step-by-step review by a qualified individual knowledgeable of the specific assays, and a written summary interpretive report must be provided to the ordering physician(s). The Thrombosis and Hemostasis will provide a written interpretation for all LA Cascade testing.

The diagnosis of APS requires both clinical and laboratory pathologic evaluations. In addition to clinical criteria, often presenting as vascular thrombosis or pregnancy morbidity, persistently positive laboratory tests are required to render a diagnosis of APA because of transient low level increase of APA in many clinical conditions including infections and reactive processes. Testing during the acute phase (i.e. at the initial presentation of thrombosis) is not recommended. Consensus guidelines suggest testing should ideally occur when the patient is not taking anti-coagulation medications.

CARDIOLIPIN AND B2-GLYCOPROTEIN 1 ANTIBODIES (IGG AND IGM)

Please note, the solid phase testing necessary to detect cardiolipin or b2-glycoprotein 1 antibodies are not included in this LA Cascade laboratory testing panel, and these assays must be ordered independently by the medical provider (Order codes CARDLI and B2PNL, respectively). These solid phase tests require serum samples and cannot be “added on” to the plasma samples used for the Lupus Cascade. Should the results from these solid phase assays be available at the time of the LA Cascade, the Thrombosis and Hemostasis Laboratory will incorporate these results into the final diagnostic interpretation.

ROUTINE COAGULATION SCREENING ASSAYS

The prothrombin time (PT) and activated partial thromboplastin time (aPTT) time are not included in this Lupus Anticoagulant Cascade. Medical providers must consider ordering these screening assays as part of their diagnostic work-up to further evaluate the possibility of other coagulation disorders.

DIRECT ORAL ANTI-COAGULANTS (DOAC)

Consensus guidelines suggest testing should only occur when the patient is free from oral anticoagulation medications including warfarin and the Direct Oral Anti-Coagulants (DOAC) medications such as dabigatran, rivaroxaban, apixaban, and edoxaban.

EXISTING LUPUS ANTICOAGULANT AND ANTI-PHOSPHOLIPID PANELS ARE DISCONTINUED

With this new Lupus Cascade, the previous panels will be discontinued:

1. Order Code APAB: DRVVT, aPTT 50:50 mix, and cardiolipin antibodies
2. Order Code LAW: DRVVT and aPTT 50:50 mix

The aPTT available at the UVMMC Laboratories is only rated moderately sensitive to the presence of a lupus anticoagulant based on the phospholipid content in the reagent system. One must consider this fact if the aPTT is utilized as a screen for a lupus anticoagulant. The new Lupus Cascade utilizes the Dilute Russell Viper Venom Test and the Silica Clot Time, both of which have increased sensitivity for detecting a lupus anticoagulant when compared to the current aPTT assay. Additionally, the Lupus Cascade will automatically perform both these tests fulfilling the recommended ISTH and CLSI guidelines of utilizing 2 phospholipid-dependent clotting assay systems.
REFERENCES:

Lupus Anticoagulant Cascade

Step 1
Perform Screening and Confirmation dRVVT and Silica Clot Time (SCT)
Both tests are NEGATIVE
LA not detected; review solid phase test results for antibodies against cardiolipin and/or β2-glycoprotein I

Step 2
SCT and/or dRVVT POSITIVE
Perform thrombin time
Prolonged
Additional heparin neutralizer and repeat thrombin time
(Perform TTHHEP if this corrects perform SCTHEP and/orDVHEP)
Prolonged (Perform FIB)
LA indeterminate; interference by thrombin inhibition
Normal
Perform mixing study
Mixing study fails to correct (SCT50 or DVV50)
Mixing study fails to correct and thrombin time normal

If the cascade is POSITIVE for a LA:
Positive results may be due to factor VIII specific inhibitor
Must confirm all 1st time positive result at 12 weeks
Must use anti-Xa for all heparin monitoring (UFH level)
Consider chromogenic FXa for warfarin monitoring

LA not detected; review solid phase test results for antibodies against cardiolipin and/or β2-glycoprotein I

Perform mixing study
Mixing study corrects
LA indeterminate; suspect factor deficiency(ies)

Perform anti-Xa assay
Anti-Xa detected
LA indeterminate: Interference by anti-Xa activity

Presumptive LA positive; confirm results with repeat testing in 12 weeks