Update to Lupus Anticoagulant Cascade Testing Algorithm

Effective June 29, 2023, the UVMMC Thrombosis and Hemostasis Laboratory updated the testing algorithm for the Lupus Anticoagulant (LA) Cascade. This updated algorithm screens for interfering anticoagulant therapy and aids in appropriate testing and/or interpretation of the LA Cascade test results. There are no changes to the way the cascade is ordered, within Epic, the orderable code remains LAB3629.

The most frequently detected antibodies in anti-phospholipid syndrome (APS) 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, ß2 glycoprotein 1 (B2GP1), cell-membrane phosphatidylserine, 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.

Consider the following before ordering LA Testing

Clinical Condition	Impact on LA Clot-Based	Recommendation
Acute event	False Positive & False Negative	1. Interpret with caution 2. Avoid testing and/or test following resolution of acute event
Anticoagulant Therapy	False Positive & False Negative	1. VKA therapy - stop at least 1 - 2 weeks prior to testing with consideration of LMWH bridging 2. If testing while on LMWH therapy, sample should be taken at least 12 hours after last dose (trough level) 3. Discontinue DOAC anticoagulation at least 48 hours prior to LA cascade testing (longer in patients with renal impairment). In patients who require ongoing anticoagulation therapy, consider switching to LMWH therapy while the patient is off DOAC therapy.
Pregnancy	False Positive & False Negative	1. Repeat testing at an appropriate time post-delivery to obtain reliable results

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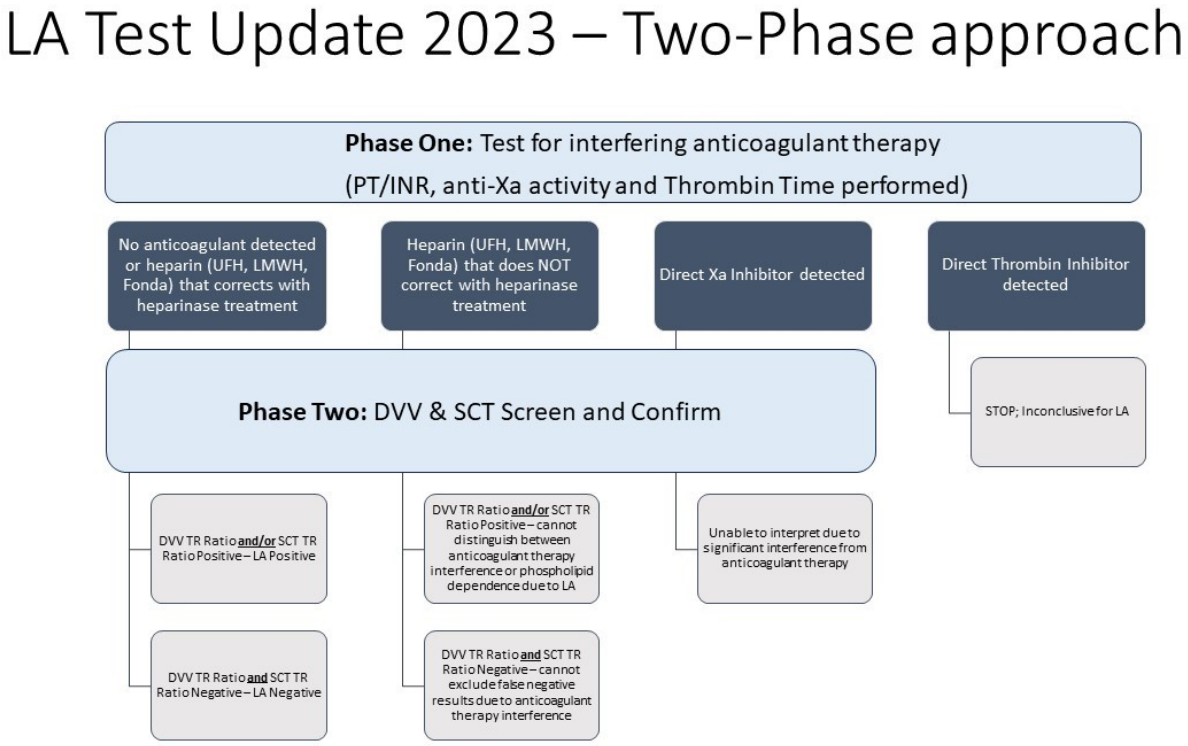
An illustration of the updated laboratory testing cascade is shown below:



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. β2-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 remind the ordering medical provider to consider the likelihood of a factor specific inhibitor in the interpretative report. Factor VIII activity assays can be performed upon request.

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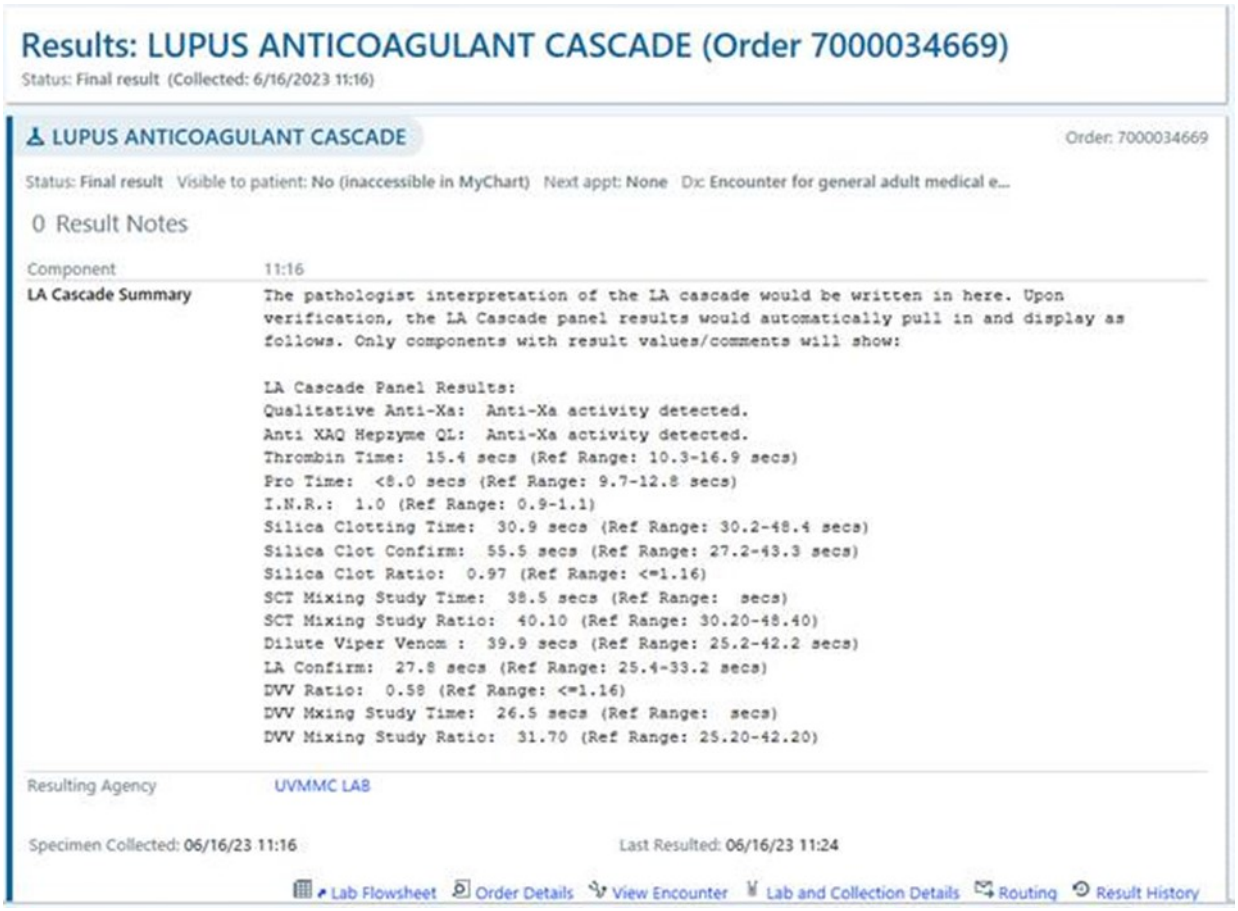
Interpretation of Laboratory Test Results

The Thrombosis and Hemostasis Laboratory will continue to provide a written interpretation for all LA Cascade testing. As part of the LA Cascade Summary interpretative report, the laboratory will also provide a new section entitled “LA Cascade Panel Results” to help aid in the review of relevant quantitative results. Only those analytes/calculations generated based on the testing cascade will be provided in the LA Cascade Panel Results. Below is an example of the LA Cascade Summary Report (all numeric values, reference ranges, etc. are for demonstration purposes and are not the correct laboratory values):



Results: LUPUS ANTICOAGULANT CASCADE (Order 7000034669)

Status: Final result (Collected: 6/16/2023 11:16)

LUPUS ANTICOAGULANT CASCADE — Order: 7000034669

Status: Final result Visible to patient: No (inaccessible in MyChart) Next appt: None Dx: Encounter for general adult medical e...

0 Result Notes

Component	11:16
LA Cascade Summary	The pathologist interpretation of the LA cascade would be written in here. Upon verification, the LA Cascade panel results would automatically pull in and display as follows. Only components with result values/comments will show: LA Cascade Panel Results: Qualitative Anti-Xa: Anti-Xa activity detected. Anti XAQ Hepzyme QL: Anti-Xa activity detected. Thrombin Time: 15.4 secs (Ref Range: 10.3-16.9 secs) Pro Time: <8.0 secs (Ref Range: 9.7-12.8 secs) I.N.R.: 1.0 (Ref Range: 0.9-1.1) Silica Clotting Time: 30.9 secs (Ref Range: 30.2-48.4 secs) Silica Clot Confirm: 55.5 secs (Ref Range: 27.2-43.3 secs) Silica Clot Ratio: 0.97 (Ref Range: <=1.16) SCT Mixing Study Time: 38.5 secs (Ref Range: secs) SCT Mixing Study Ratio: 40.10 (Ref Range: 30.20-48.40) Dilute Viper Venom : 39.9 secs (Ref Range: 25.2-42.2 secs) LA Confirm: 27.8 secs (Ref Range: 25.4-33.2 secs) DVV Ratio: 0.58 (Ref Range: <=1.16) DVV Mxing Study Time: 26.5 secs (Ref Range: secs) DVV Mixing Study Ratio: 31.70 (Ref Range: 25.20-42.20)
Resulting Agency	UVMMC LAB

Specimen Collected: 06/16/23 11:16 Last Resulted: 06/16/23 11:24

Lab Flowsheet | Order Details | View Encounter | Lab and Collection Details | Routing | Result History

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Expected Results

Please note different medical professional societies apply different criteria to determine LA Positive vs. LA Negative. For example, the **Clinical Laboratory Standards Institute (CLSI)** recommends the LA cut-off value be +2SD based on the normal local population study; however, the **International Society of Hemostasis and Thrombosis (ISTH)** recommends the LA cut-off value be at the 99%. The laboratory at UVMMC will apply the ISTH guidelines when interpreting the final **TR Ratio** results for the LA Cascade. All other values (e.g. Silica clotting time, dRVVT clotting times, etc.) will be the standard normal 95% reference interval based on the CLSI C28-A3 referenced in the vendor's package insert.

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 (measured at ≥ 12 weeks apart) are required** to render a diagnosis of APS 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.

Routine Coagulation Screening Assays and Anticoagulant Screening Assays

The prothrombin time (PT), thrombin time (TT), and qualitative anti-Xa activity assay will be the initial testing performed in the updated Lupus Anticoagulant Cascade. The reflex-only test results, along with the initial testing results, will be included in the LA Cascade Summary. They will no longer be reported as separate, distinct results. Test build updates reflecting this change will occur at a later date.

Current Algorithm					
Testing	**Always performed?**	**Comments**	**Testing**	**Always performed?**	**Comments**
Dilute Viper Venom	Yes	Initial testing	Fibrinogen	No	Reflex-only test
Silica Clotting Time	Yes	Initial testing	THT Hepzyme	No	Reflex-only test
LA Cascade Summary	Yes	Pathology Interp.	DVV Heparin Removed	No	Reflex-only test
LA Confirm Test	No	Reflex-only test	SCT Hep Removed	No	Reflex-only test
Silica Confirm Test	No	Reflex-only test	DVV Confirm Hepzymed	No	Reflex-only test
50/50 Mix DVV	No	Reflex-only test	SCT Confirm Hepzymed	No	Reflex-only test
50/50 Mix for SCT	No	Reflex-only test	Qualitative Anti Xa	No	Reflex-only test
Thrombin Time	No	Reflex-only test			

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Updated Algorithm					
Testing	**Always performed?**	**Comments**	**Testing**	**Always performed?**	**Comments**
Protime	Yes	***New Initial testing***	DVV Confirm Mix Ratio	No	Reflex-only test
INR	Yes	***New Initial testing***	SCT Screen Mix Time	No	Reflex-only test
Thrombin Time	Yes	***New Initial testing***	SCT Screen Mix Ratio	No	Reflex-only test
Qualitative Anti Xa	Yes	***New Initial testing***	SCT Confirm Mix Time	No	Reflex-only test
LA Cascade Summary	Yes	Pathology Interp.	SCT Confirm Mix Ratio	No	Reflex-only test
DVV TR Ratio	No	Reflex-only test	Dilute Russell Viper Venom Time	No	Reflex-only test
SCT TR Ratio	No	Reflex-only test	Dilute Russell Viper Venom Time Confirm	No	Reflex-only test
DVV Screen Mix Time	No	Reflex-only test	Silica Clotting Time	No	Reflex-only test
DVV Screen Mix Ratio	No	Reflex-only test	Silica Clotting Time Confirm	No	Reflex-only test
DVV Confirm Mix Time	No	Reflex-only test	Qualitative Anti Xa Hepzymed	No	Reflex-only test

The LA Cascade CPT codes have been updated as follows:

Initial Testing	CPT Code(s)
Protime/INR	85610
Thrombin Time	85670
Qualitative Anti Xa	85520
LA Cascade Summary	85390.26

The CPT codes for the potential reflex-only testing are as follows:

Reflex-only Testing	CPT Code(s)
DVV TR Ratio	N/A
SCT TR Ratio	N/A
DVV Screen Mix Time	85613
DVV Screen Mix Ratio	N/A
DVV Confirm Mix Time	85613
DVV Confirm Mix Ratio	N/A
SCT Screen Mix Time	85732
SCT Screen Mix Ratio	N/A
SCT Confirm Mix Time	85732
SCT Confirm Mix Ratio	N/A
Dilute Russell Viper Venom Time	85613
Dilute Russell Viper Venom Time Confirm	85613
Silica Clotting Time	85732
Silica Clotting Time Confirm	85732
Qualitative Anti Xa Hepzymed	85520, 85525

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Medical providers must consider ordering other routine and specialized coagulation 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 DOAC medications such as dabigatran, rivaroxaban, apixaban, and edoxaban.

Cardiolipin and β2-glycoprotein 1 antibodies (IgG and IgM)

Please note, the solid phase testing necessary to detect cardiolipin or β2-glycoprotein 1 antibodies is not included in this LA Cascade laboratory testing panel, and these assays must be ordered independently by the medical provider (Epic Codes: LAB464 and LAB2335, respectively). These solid phase tests require serum samples and cannot be "added on" to the plasma samples used for the Lupus Cascade.

For questions or concerns regarding these changes, please contact Dr. Sarah Harm at Sarah.Harm@uvmhealth.org.

References

Devresse K, Groot P, et al. Guidance from the Scientific and Standardization Committee for lupus anticoagulant antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis - Update of the guidelines for lupus anticoagulant detection and interpretation. J *Throm Haemost*. 2020;18:2828-2839.

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Vandevelde A and Devreese Katrien. Laboratory Diagnosis of Antiphospholipid Syndrome: Insights and Hindrances. *J Clin Med.* 2022;11: 2164.

Tripodi A, Cohen H, et al. Lupus anticoagulant detection an anticoagulated patients. Guidance from the Scientific and Standardization Committee for lupus anticoagulant /antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost.* 2020;18:1569 – 1575.

Moore G. Recent Guidelines and Recommendations for Laboratory Detection of Lupus Anticoagulants. *Semin Thromb Haemost.* 2014;40:163 – 171.

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