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Laboratory Testing for the Lupus Anticoagulant; Approved Guideline

This document provides guidance and recommendations regarding the proper collection and handling of the specimen; descriptions and limitations of screening and confirmatory assays, and mixing tests used to identify lupus anticoagulant (LA); determination of cutoff values and calculations associated with the various assays; and interpretation of test results in an LA panel.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Laboratory Testing for the Lupus Anticoagulant; Approved Guideline

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Abstract

Identification of the lupus anticoagulant (LA) by laboratory testing is critical for diagnosing the antiphospholipid syndrome and investigating unexpectedly prolonged activated partial thromboplastin time values. The “anticoagulant” effect of LA is restricted to the prolongation of clotting times when using *in vitro,* clot-based coagulation assays that are used as surrogates for identifying LA. Clinical and Laboratory Standards Institute document H60—*Laboratory Testing for the Lupus Anticoagulant; Approved Guideline* provides guidance and recommendations regarding the proper collection and handling of the specimen; descriptions and limitations of screening and confirmatory assays, and mixing tests used to identify LA; determination of cutoff values and calculations associated with the various assays; and interpretation of test results in an LA panel. The guideline is provided for use by laboratorians, physician stakeholders, manufacturers of LA assays, researchers, external quality assessment programs, and accrediting and regulatory agencies. The intent of this guideline is to present information in a practical and easily understandable format; thereby facilitating a standardized approach to LA testing, gaining acceptance in practice, and improving testing quality.

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The authors would like to dedicate this document to the memory of Douglas A. Triplett, MD, whose work in lupus anticoagulant testing and the antiphospholipid syndrome has left an indelible mark. His innumerable contributions through scientific research and education have guided many in this field. Dr. Triplett's name is synonymous with lupus anticoagulant testing and we are indebted to him for his efforts and commitment to this field.

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Foreword

Synopsis of Diagnostic Criteria and Testing Recommendations

Criteria for the Laboratory Diagnosis of the Lupus Anticoagulant

In order to make a laboratory diagnosis of the lupus anticoagulant, a sample should identify with the following:

A. **Procurement:** adherence to standardized protocols for collection and processing of blood to be used for testing

B. **Screening:** prolongation of at least one of two different phospholipid-dependent clotting assays based on different principles and coagulation pathways

C. **Confirmation:** evidence that prolongation of the screening test(s) demonstrates phospholipid dependence by using a similar second test(s) using altered concentrations and/or composition of phospholipids

D. **Mixing:** if mixing assays are performed, evidence of inhibitory activity shown by the effect of patient plasma on an equal volume of normal pooled plasma

E. **Exclusion:** distinguishing the lupus anticoagulant from other causes of prolonged clotting times that may mask, mimic, or coexist with the lupus anticoagulant, such as anticoagulant therapies or other coagulopathies

F. **Interpretation and Reporting:** numerical results of all testing should be reported, and interpretive comments that address and integrate these results should be provided

Recommendations Specific to Each Criterion for the Laboratory Diagnosis of the Lupus Anticoagulant

A. **Procurement**

1. Testing should preferably be performed in the absence of anticoagulant therapy (except for antiplatelet therapy).

2. Ideally, samples should not be obtained from vascular access devices.

3. Platelet count of patient-citrated platelet-poor plasma should be $< 10 \times 10^9$/L.

4. Testing may be performed on fresh or properly frozen/thawed samples.

B. **Screening Assays**

1. Two tests, representing different principles and coagulation pathways, that are known to be responsive to the lupus anticoagulant (eg, low phospholipid concentrations) should be used to screen for the lupus anticoagulant.

2. Lupus anticoagulant–responsive activated partial thromboplastin time and dilute Russell's viper venom time tests are recommended as the preferred minimal screening assays.

3. Other tests for the lupus anticoagulant referenced in this document may supplement the preferred minimal screening tests.

4. Where test design permits, results should be calculated using the mean of the reference interval and reported as a normalized ratio.

5. Routine coagulation tests, prothrombin time-international normalized ratio, activated partial thromboplastin time, and thrombin time, as indicated, may help to characterize anticoagulant effects (eg, heparin, vitamin K antagonists, direct thrombin inhibitors, factor Xa inhibitors) or sample suitability (eg, serum sample, improper anticoagulant tube) for lupus anticoagulant testing and interpretation.

C. **Confirmatory Assays**

1. Confirmatory assays should use the same assay principle as the screening test that was initially found to be abnormal (eg, if the dilute Russell's viper venom time test is abnormal, then a dilute Russell's viper venom time test–based confirmatory assay should be used).

2. For paired tests, results should be calculated using the mean of the reference interval for each screening and confirmatory test and reported as a normalized screen to confirm ratio or indication of percentage correction of screen ratio by confirm ratio.

3. Solid-phase immunoassays for antibodies against phospholipid (eg, anti-cardiolipin or anti-β2 glycoprotein I) should not be considered as lupus anticoagulant confirmatory procedures.

D. **Mixing Test** (if performed)

1. The platelet count of the normal pooled plasma should be $< 10 \times 10^9$/L.

2. A mix ratio of one part plasma sample to one part normal pooled plasma is recommended as the preferred ratio for a mixing test.

3. The dilution effect of a 1:1 mixing test may mask lupus anticoagulant inhibitory activity. Other mix ratios (eg, four parts plasma sample to one part normal pooled plasma) can be used, if validated by the laboratory.

4. Mixing test inhibition is assessed by either comparison of normalized ratios to cutoff values specific for each lupus anticoagulant screening or confirmatory mixing test or by calculating an index of circulating anticoagulant.

5. Incubated mixing tests are not recommended for routine lupus anticoagulant testing, but should be performed when indicated (eg, when a specific factor inhibitor is suspected).

E. **Exclusion**

1. The lupus anticoagulant should be distinguished from anticoagulant therapies and/or other coagulation disorders that may interfere with lupus anticoagulant testing and interpretation.

2. If possible, perform factor assays whenever there is suspicion of a specific factor deficiency or inhibitor, using three or more dilutions of patient plasma and an activated partial thromboplastin time reagent that is unresponsive to the lupus anticoagulant.

F. **Interpretation and Reporting**

1. Numerical results of all testing should be reported with the reference interval or cutoff value.

2. Interpretive comments that address and integrate all test results (the lupus anticoagulant panel) should be provided.

3. The interpretive report should indicate whether the lupus anticoagulant is present, not detected, or indeterminate.

4. Solid-phase assays for antibodies against cardiolipin and/or anti-β2 glycoprotein I are recommended as part of an evaluation for antiphospholipid syndrome.

5. If the lupus anticoagulant is present, the test panel should be repeated at or beyond 12 weeks to determine persistence of the lupus anticoagulant as part of the evaluation for antiphospholipid syndrome.

Key Words

Antiphospholipid syndrome, lupus anticoagulant, lupus anticoagulant confirmatory assays, lupus anticoagulant screening assays, mixing test

Laboratory Testing for the Lupus Anticoagulant; Approved Guideline

1 Scope

This document provides guidance for the performance and interpretation of screening assays, confirmatory assays, and mixing tests used to identify the lupus anticoagulant (LA). It is intended to assist in standardization of LA testing and it addresses preexamination issues, examination concerns, and postexamination matters that pertain to interpretation of individual tests or combinations of assays. Recommendations from this guideline, when feasible, harmonize with other national and international guidelines currently in existence. Taken together, standardization and harmonization will permit laboratories to improve the quality and interpretation of their LA testing.

The intended users of this guideline are laboratory personnel responsible for performing LA testing, physicians (eg, hematologists, pathologists, rheumatologists), external quality assessment (EQA) programs, researchers, and manufacturers of reagents used in LA testing.

Two types of methodologies are used for the diagnosis of the antiphospholipid syndrome (APS). This guideline is limited to clot-based coagulation assays used as surrogates for identifying LA—a strong risk factor for thrombosis. The guideline will not address solid-phase testing for anti-phospholipid (aPL) (eg, anti-cardiolipin [aCL] or anti-β2 glycoprotein I [aβ2GPI]), because detection of these specific antibodies may or may not relate to the laboratory anomaly of a prolonged activated partial thromboplastin time (APTT).

2 Introduction

Identification of LA by laboratory testing is critical for investigating unexpectedly prolonged APTT values and diagnosing APS. According to recent consensus classification criteria, two conditions must be met for defining APS: 1) the **persistent** presence of circulating aPL in plasma and/or serum, and 2) a history of thrombosis and/or pregnancy morbidity including fetal loss.[1,2] APS is an autoimmune disorder that occurs in patients who, in general, also show laboratory evidence of antibodies directed against plasma proteins that have an affinity for anionic phospholipids (PL). The dominant antigenic targets recognized by aPL in patients with APS are β2GPI or prothrombin.[3-5]

The terms APS and LA are both misnomers and LA itself is a double misnomer.[6,7] The autoantibodies associated with APS are not directed against PL in general but specifically against proteins that bind to anionic PL. LA comprises a heterogeneous group of autoantibodies that can develop in individuals with autoimmune conditions (systemic lupus erythematosus [SLE], APS, or other autoimmune disorders) or can arise spontaneously.[8-10] LA can also be found transiently in plasma from patients with infections or malignancies, or from patients using certain drugs. The "anticoagulant" effect of LA is restricted to the prolongation of clotting times (competition between these antibodies and coagulation proteins for PL surfaces) in clot-based assays (ie, *in vitro*), whereas *in vivo*, these antibodies are variably associated with thrombosis.[11,12] Persistent LA is the most important acquired risk factor for thrombosis (or its recurrence) in APS.[8,13] LA may also cause bleeding due to immune-mediated deficiencies of coagulation factors II (FII) or X (FX) (see Section 7.1.1). Infection- or drug-induced LA tend to be transient, disappearing after the infection resolves or when the medication is discontinued. Transient LA due to infections have rarely been reported to increase thrombotic risk in association with immune-mediated protein S deficiency (see Section 7.1.1). LA due to certain medications may not be transient and can increase thrombotic risk.[14] LA associated with malignancy might also resolve after the malignancy is treated.[15,16]