Lupus Anticoagulant Confirmation Reagent

REF 102516

English

PRODUCT DESCRIPTION

Lupus Anticoagulant Confirmation Reagent is a lyophilized preparation of phosphatidyl enhanced platelet phospholipid.

INTENDED USE

Lupus Anticoagulant Confirmation Reagent is the third test component, phosphatidyl platelet phospholipid solution, used after and with other IVD reagents to confirm the prior three previous tests correctly flagged a sample as one containing the lupus anticoagulant.

PRINCIPLE

The most common cause of an UNDETERMINED prolonged APTT is the presence of the lupus anitcoagulant in the plasma. The inhibitory activity is demonstrable in phospholipid dependent coagulation reactions, but is observed more often in the APTT than in the Prothrombin Time (PT). The addition of Lupus Anticoagulant Confirmation Reagent will shorten the APTT time that is prolonged in the presence of the lupus anticoagulant. Thus, the platelet neutralization procedure system is a valuable diagnostic test to differentiate lupus anticoagulant from a specific factor inhibitor (e.g. Factor VIII inhibitor).[5]

COAGULATION PROCESSES

Figure 1A

Interruption of the Traditional Coagulation Cascade by Antiphospholipid Antibodies

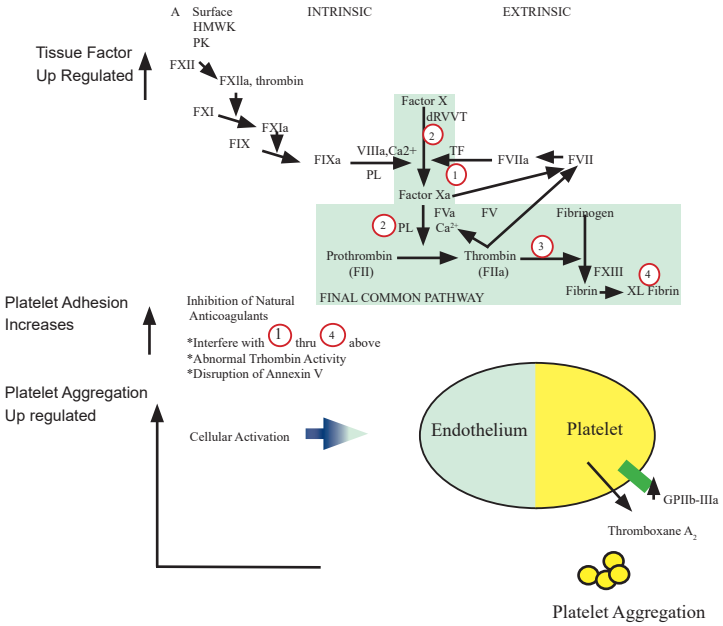


*Figure 1A: Utz, VM and Tang, J. Ocular Manifestations of the Antiphospholipid Syndrome. Br J Ophthalmol. Doc 10.1136/bjo.2010.182857.

FIGURE 1B

Phospholipid dependent reactions are blocked by the action of antiphospholipid antibodies at two key points.

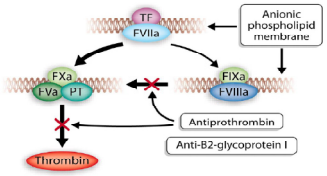
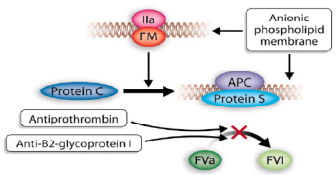


FIGURE 1C

Factor Va inactivation (control regulation) by inhibition of Protein C mechanism.



Figures 1B and 1C: Hill, GS and Nochy, D. Antiphospholipid Syndrome in Systemic Lupus Erythematosis. J AM Soc Neph. 18: 2461 – 2464. 2007

An anticoagulant which prolongs the activated partial thromboplastin time (APTT) and occasionally the prothrombin time (PT) of otherwise normal plasma, but does not specifically inactivate any of the known clotting factors, has been designated as the "lupus" anticoagulant (LA).[6]

Laboratory diagnosis of the lupus anticoagulant is based on the presence in plasma of inhibitory activity in phospholipid-dependent clotting tests. In addition to the PT and the APTT, other tests for LA have included the tissue thromboplastin inhibition procedure (TTI)[7], kaolin clotting time (KCT)[8], and recalcified clotting time of platelet-rich plasma (PCT)[9]. Additionally, use of the dilute Russell Viper Venom Time (RVVT)[10] and the dilute phospholipid APTT assay[11] have been described. Several of these procedures have subsequently led to definitive studies of the role of phospholipids in the assessment of the presence of the lupus anticoagulant in plasma[12].

In this regard, Triplett et al introduced the platelet neutralization procedure (PNP) in 1983 [1]. They noted that the inhibitory effect of the lupus anticoagulant is neutralized by products of platelet lysis. Further, the platelet neutralization procedure appears useful in differentiating the lupus anticoagulant from factor specific inhibitors. The APTT of a lupus plasma is shortened whereas the result of a factor specific inhibitor plasma is not shortened. The test is highly specific in this regard, and has been alluded to or confirmed in studies described in recent reports. [9, 12, 13, 14, 15]

PRECAUTIONS

Lupus Anticoagulant Confirmation Reagent is for PROFESSIONAL LABORATORY USE ONLY AND IN-VITRO DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION. The platelets have been tested at the source and found to be negative for HIV-1Ag, anti-HIV-1/2, Hepatitis B surface antigen, Hepatitis C antibody, Human T-Lymph tropic Type I and II (anti-HTLV I/II) and negative by a serological test for Syphilis. However, all plasma and platelets of human origin should be handled as being potentially hazardous.

NOTE TO USER: Any serious incident that occurs in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

MATERIALS PROVIDED

Lupus Anticoagulant Confirmation Reagent, 5 x 1.0mL. Store at 2° to 8°C prior to reconstitution.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Coagulation Analyzer: Optical, Automated, Semi-Automated or Manual
2. Purified water (distilled, deionized or reagent grade), pH 5.3 - 7.2
3. Pipettors (0.1mL, 0.6mL, 1.0mL volumes)
4. Activated partial thromboplastin reagent (mirosilica activator)
5. Calcium chloride, 0.025 M
6. Normal plasma control
7. TRIS Buffered Saline, 0.06 M, pH 7.5 or 0.85% (w/v) Saline

INSTRUMENTATION

The Platelet Neutralization Procedure may be performed manually or on any automated or semi-automated coagulation instrument. Follow the manufacturer's instructions for operating the instrument in use.

SPECIMEN COLLECTION AND PREPARATION OF TEST PLASMA

Proper specimen collection, labeling, transport and processing are critical steps for coagulation tests. Coagulation specimens should be collected, transported and prepared in accordance with CLSI guidelines[4]. Use evacuated specimen collection tubes with an inner plastic tube or silicone coating. Plastic syringes may also be used.

1. **PATIENT PREPARATION:**
   For baseline studies, patients should fast and avoid fatty foods for 12 hours prior to specimen collection. Fasting is not required for subsequent specimens.

2. **SPECIMEN COLLECTION:**
   Blood collection should be performed with care to avoid stasis, hemolysis and contamination by tissue fluids. Test plasma should be prepared from whole blood specimens collected in a 0.11 M buffered citrate anticoagulant.[4]

   Evacuated Collection Tube Technique.
   1. Use a winged needle for the venipuncture.
   2. Draw blood using (plastic) tubes containing 0.11 M Sodium Citrate anticoagulant. Keep tube capped until after the centrifugation process is completed.
   3. Gently invert 4-5 times to mix the blood and anticoagulant.

If the patient's hematocrit is <30% or >50%, the blood to anticoagulant level must be adjusted. Contact the laboratory for instructions.[4]

If testing is delayed, refrigerate the plasma (2° to 8°C for a maximum of 2 hours). Beyond 2 hours, freeze the plasma at -20°C or lower. (Frozen plasma may not be stable for all coagulation factors).[4]

Evacuated tube cap color does not distinguish buffered citrate concentrations. Check the label to confirm the proper tube is used.

Syringe Technique
1. Using a winged needle perform a traumatic venipuncture.
2. Draw 9.0mL of blood into a plastic syringe. Avoid excess suction.
3. Remove the needle from the syringe and immediately and gently dispense the blood into a plastic tube containing Sodium Citrate (0.11M) anticoagulant. Ratio of Blood to anticoagulant must be 9 parts of blood to 1 part anticoagulant (3.2% Citrate).
4. Cover and invert 3-4 times gently to mix.

Observe standard precautions throughout specimen collection, sample preparation and analytical processes. [2,3] Biological waste must be disposed of in accordance with laboratory policy.

PREPARATION OF PLATELET FREE PLASMA (PFP)

1. Prepare the platelet poor plasma by centrifuging the remaining blood specimen for at 2500 x g for 20 minutes. Residual platelet count must be less than 5,000/mm.[3]
2. Remove plasma from cells with a plastic transfer pipette, being careful not to disturb the buffy coat. Transfer the plasma to a plastic vial and cap it. Plasma should be free of red cells and platelets.

NOTE: Test plasma with platelet counts greater than 5,000 per ul may normalize certain test results and should be recentrifuged prior to testing and freezing.

RECONSTITUTION
NOTE: Reagents must be at room temperature (15° to 28°C) prior to reconstitution. Stored reagent must be brought to room temperature prior to use.

Reconstitute a vial of Lupus Anticoagulant Confirmation Reagent with 1.0mL purified water.

REAGENT STORAGE
The reconstituted Lupus Anticoagulant Confirmation Reagent is stable for 30 days when stored at 2° - 8° C in its original tightly sealed container.

TEST PROCEDURE
The platelet neutralization procedure (PNP) assay is based on the microsilica activator based activated partial thromboplastin time (APTT) technique. IT SHOULD BE NOTED THAT THE VARIABILITY IN APTT REAGENTS FROM DIFFERENT MANUFACTURERS CAN HAVE AN EFFECT ON THE PNP. [16-22] The APTT methodology currently in use in the laboratory should be followed.

PART 1: ROUTINE BASELINE APTT
1. Pipette 1 part of normal control plasma into a test cuvette.
2. Add 1 part of APTT reagent to the control plasma. Mix well.
3. Incubate the plasma-APTT mixture for the activation time recommended by the reagent manufacturer.
4. Add 1 part of prewarmed 0.025 M calcium chloride, simultaneously starting the timer.
5. Record the clotting time.
6. Repeat steps 1-5 for a duplicate sample. Duplicate results should correlate within ± 5%.
7. Record results for final evaluation.
8. Repeat steps 1-7 for each test plasma.

PART 2: PLATELET NEUTRALIZATION PROCEDURE
1. Pipette 1 part normal control plasma into a test cuvette.
2. As a dilution control, add 0.1mL TRIS Buffered Saline, 0.06 M pH 7.5 or 0.85% (w/v) saline.
3. Add 1 part APTT reagent, mix well.
4. Incubate for recommended activation time, as in Part 1, Step 3.
5. Add 1 part prewarmed calcium chloride, simultaneously starting the timer.
6. Repeat steps 1 - 5 for a duplicate sample. Duplicate results should correlate within ± 5%. Record saline/normal control plasma results for final evaluation.
7. Repeat steps 1 - 6 for each test plasma. Record saline control for each test plasma.
8. Pipette 1 part normal control plasma into test cuvette.
9. Add 1 part of reconstituted Lupus Anticoagulant Confirmation Reagent.
10. Add 1 part of APTT reagent. Mix well.
11. Incubate for recommended activation time.
12. Add 1 part prewarmed calcium chloride, simultaneously starting timer.
13. Repeat steps 8 - 12 for duplicate sample. Duplicate results should correlate within ± 5%. Record Lupus Anticoagulant Confirmation Reagent/normal control plasma results for final evaluation.
14. Repeat steps 8 -13 for each test plasma. Record each Lupus Anticoagulant Confirmation Reagent/ test plasma result for final evaluation.

QUALITY CONTROL
Performance of platelet neutralization procedure on normal control plasma is necessary to provide a reference point for interpretation of patient test results. The control should be assayed exactly as the test plasma. APTT values should be ascertained on a 1:1 mixture of saline/normal control plasma and a 1:1 mixture of Lupus Anticoagulant Confirmation Reagent/ normal control plasma.

RESULTS AND FINAL EVALUATION
The clotting time of the Lupus Anticoagulant Confirmation Reagent/test plasma mixture is compared to its saline/test plasma control. A correction of the prolonged APTT of 5 seconds or greater for the Lupus Anticoagulant Confirmation Reagent/test plasma mixture as compared to the saline/ test plasma control, is considered a positive test result.

EXPECTED VALUES
Expected Ranges for coagulation testing should be established by each laboratory.

CORRECTION DETERMINATION
Subtract the LACR APTT time (secs) from the saline APTT time (secs) to determine the correlation

Saline Test Plasma APTT - LACR Test Plasma = correction

An example of three correction study panels is illustrated:

Patient	Baseline APTT (sec)	APTT (sec) saline/test plasma	APTT (sec) LA Conf Rgt /test plasma	Difference between APTT/LA Conf Rgt & APTT/saline	Eval.
1	49	44	43	1	Neg.
2	50	53	52	1	Neg.
3	75	53	41	12	Pos.

Patients 1 and 2 are reported as negative for the lupus anticoagulant. Patient 1 results are indicative of a specific factor inhibitor rather than a lupus anticoagulant because the APTT diluted with Lupus Anticoagulant Confirmation Reagent and the APTT diluted with saline BOTH corrected from the baseline APTT to the same extent. Here, correction is attributed to dilution of an inhibitor. Patient 2 APTT test results showed no correction with the addition of the Lupus Anticoagulant Confirmation Reagent or saline. The lack of correction is interpreted as negative for the lupus anticoagulant and more indicative of the presence of a factor deficiency rather than an inhibitor. Patient 3 results are indicative of the presence of a lupus anticoagulant. Refer to the flow chart for instructions on the evaluation of a prolonged APTT.

It should be noted that expected values may vary from laboratory to laboratory due to the heterogeneity of laboratory results in patients with the lupus anticoagulant.[16] Additionally, differences in coagulation results have been described to occur due to sensitivity and responsiveness of activated partial thromboplastin time reagents.[17,18,19, 20] Parameters for expected values should be established by each laboratory.

LIMITATIONS
Lupus Anticoagulant Confirmation Reagent is a phosphatidyl enhanced platelet phospholipid used to determine if a prolonged APTT is caused by a lupus inhibitor or another coagulation factor deficiency.[19] Lupus anticoagulants demonstrate considerable heterogeneity and show variable differences in sensitivity and responsiveness to the APTT reagent. The sensitivity and responsiveness may vary from lot to lot of APTT reagent. The quality of a result can only be as good as the quality of the specimen. To avoid erroneous results, specimen should be free of platelets and hemolysis.

Clinical history is required for accurate test interpretation. Patients should be questioned about the recent ingestion of any medication because a number of prescription and non-prescription drugs may interfere with coagulation. Dose and time taken should be noted for patients on Coumadin®.

PERFORMANCE CHARACTERISTICS
The Lupus Anticoagulant Confirmation Reagent has been tested on plasmas of patients with a lupus anitcoagulant. Studies have shown that the shortening effect on the APTT may vary with the titer of the inhibitor in the test plasma.

Studies have shown that this product will perform as described when procedural and storage directions are followed.

Linearity
The complex chemistry and nature of measurement of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) testing is not a linear relationship.
Clotting times do not change in a linear ratio to the clotting factor(s) that are being measured.
The limits of the instrument and method dictate the linearity of this assay

Accuracy, Precision and Reproducibility
The accuracy, precision and reproducibility of coagulation testing are technique and instrument dependent. The Laboratory should establish its own limits of acceptability based on written laboratory protocols and accepted laboratory standards.

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