Lupus Anticoagulant Confirmation Reagent

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). 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). 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). 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).

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)

COAGULATION PROCESSES

Figure 1A

Interruption of the Traditional Coagulation Cascade by Antiphospholipid Antibodies

Platelet Adhesion

Increases

Platelet Aggregation

Up regulated

Cellular Activation

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.

Materials provided:

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

Precautions:

Lupus Anticoagulant Confirmation Reagent is for 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-1, 2, anti-HIV-1/2, Hepatitis B surface antigen, Hepatitis C antibody, Human T-Lymphotropic Virus 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.

Materials required but not provided:

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

Instruments:

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. 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.

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.

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).

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 venepuncture.
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 level must be adjusted. Contact the laboratory for instructions.

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).

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

Observe standard precautions throughout specimen collection, sample preparation and analytical processes. 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.
2. Resuspend 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 neutralize certain test results and should be refrezen 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.
Thromboplastin Time (APTT) testing is not a linear relationship. 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.

REFERENCES

4. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.