HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

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| **Department:** | Laboratory and Blood Bank (Hematology) | | |
| **Document:** | Internal Policy and Procedure | | |
| **Title:** | Lupus Anticoagulant | | |
| **Applies To:** | All Hematology Staff | | |
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## 1. PURPOSE:

1.1 This describes the procedure for the detection of lupus anticoagulant (LA), in plasma by PTT-LA (Activated partial thrombopalstin time sensitized to aid the detection of lupus anticoagulant applied as a screening test).

## 2. DEFINITIONS:

2.1 Lupus anticoagulants (LA) are auto-antibodies against negatively charged phospholipids or complex of phospholipids with either beta-2-glycoprotein 1 or clotting factors such as prothrombin. They occur in various clinical conditions, especially autoimmune diseases. LA have traditionally been detected using phospholipids responsive clotting tests, such as activated partial thrombopalstin time (APTT), Kaolin clotting time (KCT).

## 3. POLICY:

3.1 PTT-LA: The principle of PTT-LA test is based on the measurement of plasma recalcification time in the presence of cephalin and activator. The presence in the test plasma of lupus anticoagulant prolongs the clotting time. Sensitization of the reagent specifically enhances the prolongation of the clotting time due to the LA in the test plasma.

## 4. PROCEDURE:

4.1 **Specimen collection:**

4.1.1 Collect the blood in a blue stopper vacutainer tube using a ratio of 9 parts of whole blood and 1 part of 3.2% buffered sodium citrate.
4.1.2 Centrifuge the specimen at no less than 3500 rpm for 10 minutes.
4.1.3 Test the supernatant, with patient demographic identification. For the best results, test must be performed immediately.
4.1.4 Freeze the plasma at -20°C or lower until ready to test.
4.1.5 Frozen plasma must be thawed at 37°C for 10 minutes directly before testing.
4.1.6 Specimens are stable for 4 hours at 18°-24°C or 2°- 4°C and 2 weeks at -20°C.

4.2 **Procedural steps:**

4.2.1 STA- Compact Operation and maintenance procedure to help you in performing the assay.
4.2.2 No calibration is needed.

4.3 **Quality Control:**

4.3.1 STA- Control 1.
4.3.2 STA- Control 2.
4.3.3 Check values to ensure that results are within acceptable limits.
4.3.4 Patient results cannot be released if the run is rejected based on Westgard Rules.
4.3.5 Check reagents and controls for expiration date.
4.3.6 For Staclot LA20 and PTT-LA have special manual way to be entered on Stago according to the following table:

| ID | NAME | VOLUME(ml) | LOT # | POSITION | STIRRING bar |
|---|---|---|---|---|---|
| PTT-LA | PTT-LA | 2.0 | 2.0 | 111111 | -------- |
| 111111 | LA neg | 1.0 | 1.0 | 123 | -------- |
| 222222 | LA pos | 1.0 | 1.0 | 124 | -------- |

****** Should PTT-LA be mixed- NO******

4.3.6.1 Results of ≥45 sec are considered POSITIVE

4.3.6.2 Proceed to confirmation by running Staclot-LA test.

4.3.7 Staclot-LA: Staclot LA1, Staclot LA2

Note: all the reagents will be placed in micro tubes except for the phospholipids which will be placed in special holder with magnetic stirring bar.

| I.D | Name | Volume (mL) | Stable hours | Lot# | Position | Stirring bar |
|---|---|---|---|---|---|---|
| Reagent 1 | Reagent 1 | 1 | 8 | 1111 | ---------- | No |
| Reagent 2 (Phospholipids with magnetic | Reagent 2 | 1 | 8 | 2222 | ---------- | Yes |
| Reagent 3 | Reagent 3 | 1 | 8 | 3333 | ---------- | No |
| PTT-LS | PTT-LS | 1 | 8 | 4444 | ---------- | No |
| 11111 | LA Negative | 1 | 8 | 123 | ---------- | No |
| 22222 | LA Positive | 1 | 8 | 124 | ---------- | No |

C. Final Step:

- If LA1 – LA2 < 8 sec ➡ Negative.
- If LA1 – LA2 ≥ 8 sec ➡ Positive.

4.3.8 Expected values and interpretation of results:

LA1 (Screen), Pool Norm are tested together, and screen ratio is calculated using this equation:

$$\text{Screen Ratio} = \frac{\text{Screen Ratio Patient Plasma Clotting Time}}{\text{Reference normal pool clotting time}}$$

4.3.9 When the Screen Ratio of the patient is < 1.2, the patient is unlikely to have LA anti-coagulant. But if the screen ratio is ≥1.2, the result is abnormal and the presence of LA is suspected.

4.3.10 PTT-LA is tested for each sample after the controls done. if P T T-LA < 45 sec, the patient is unlikely to have lupus anticoagulant

4.3.11 If Staclot LA-20 < 8 sec, the patient is unlikely to have lupus anticoagulant, but if Staclot LA-20 ≥ 8 sec the presence of LA is confirmed.

4.3.12 When the screen ratio is ≥1.2, perform mixing studies using the reference normal pool, to exclude acoagulant factor deficiency (if LA Ratio is corrected after the mixing of the Normal Pool) or vitamin K antagonist therapy from a circulating anticoagulant. (perform the clotting time after the mixing of the normal pool with the patient plasma, even if the result is corrected, you have to incubate for 2 hours).

4.3.13

$$\text{Rosner index (Mixing study formula):} \quad \frac{\text{Mix (P+ N) CT} - \text{Normal plasma CT}}{\text{Patient plasma CT}}$$

(P= Patient, N= normal plasma pool, CT= clotting Time)

Ratio < 12 Negative

Ratio between 12- 15 Suspicious Do LA Confirm.

Ratio ▯ 15 Positive Do LA Confirm.

LA2 (Confirm), Pool Norm are tested together, confirm ratio and normalization ratio are calculated using these equation:

$$\text{Confirm Ratio} = \frac{\text{Patient Plasma Clotting Time (LA2)}}{\text{Patient plasma CT}}$$

$$\text{Normalization Ratio} = \frac{\text{Screen Ratio (LA1)}}{\text{Confirm Ratio (LA2)}}$$

4.3.14 When the Normalization Ratio of the patient is < 1.2, the patient is unlikely to have LA anti-coagulant and it could be a factor inhibitor. But if the Normalization Ratio is ≥1.2, the result is abnormal and the LA anticoagulant is most likely present.

4.3.15 Persistence of the positive result test must be demonstrated in a separate blood sample collected at least 12 weeks a part.

4.3.16 Clinical indications:

- 4.3.16.1 Suspected phospholipids antibodies (e.g. prolonged aPTT).
- 4.3.16.2 Screening for LA in thrombotic disease.
- 4.3.16.3 Screening for LA in case of repeated miscarriages.
- 4.3.16.4 Screening for thrombophilia

## 5. MATERIALS AND EQUIPMENT:

5.1 Reagent preparation and storage:

5.1.1 **PTT-LA:**

Reconstitute each vial of PTT-LA with 2 mL of distilled water. Allow it to stand at room temperature (18°-25°C) for 30 minutes, then swirl the vial gently to obtain homogenous solution.

5.1.2 **S TA- control LA 1+ 2:**

Reconstitute the lyophilized powder with 1 ml of distilled water. Allow it to stand at room temperature (18°-25 °C) for 30 minutes. Mix reagent well by swirling the vial without creating any bubbles before use.

5.1.3 **Pool Norm**. (Reference normal pool):

Reconstitute the lyophilized powder with 1 ml of distilled water. Allow it to stand at room temperature (18°-25 °C) for 30 minutes. mix reagent well by swirling the vial without creating any bubbles before use

5.1.4 **Staclot LA 20**:

Which is reagent system designed for the quantitative detection of LA in plasma by the use of hexagonal H11 phase- phospholipids molecules. This kit includes 5 reagents:

- 5.1.4.1 Reagent 1: Ready for use buffer
- 5.1.4.2 Reagent 2: lyophilized hexagonal phase phosophotidyllethanolamine
- 5.1.4.3 Reagent 3: lyophilized normal human plasma containing heparin inhibitor.
- 5.1.4.4 Reagent 4: lyophilized PTT-LS reagent consisting of cephalin prepared from rabbit cerebral tissues and a particular siliceous
- 5.1.4.5 Reagent 5: solvent for reconstitution of reagent 4.

5.1.5 **Storage:**
The reagent in intact vials is stable until the expiration date indicated on the box label, when stored at (+2° to +8 °C). Reconstituted reagents with, STA- mini reducer and perforated plastic cap in place, LA1 and LA2 remains stable for 72 hours on STA- Compact. For STA- control 1+ 2 they are stable for 4 hours on STA- Compact, and at room temperature (18°-25°C), 2 weeks at -20°C and 6 months at -70°C.

## 6. RESPONSIBILITIES:

6.1 This policy applies to all Hematology technologists involved in this special Hematology test

## 7. APPENDICES:

N/A

## 8. REFERENCES:

8.1 PTT-LA leaflet insert
8.2 Staclot La-20 leaflet insert

## 9. APPROVALS:

| | Name | Title | Signature | Date |
|---|---|---|---|---|
| **Prepared by:** | Dr. Fatma Hassan Ahmed | Clinical Pathologist | | January 07, 2025 |
| **Reviewed by:** | Dr. Kawther M. Abdou | Consultant & Lab. Medical Director | | January 08, 2025 |
| **Reviewed by:** | Ms. Noora Melfi Alanizi | Laboratory & Blood Bank Director | | January 09, 2025 |
| **Reviewed by:** | Mr. Abdulelah Ayed Al Mutairi | QM&PS Director | | January 12, 2025 |
| **Reviewed by:** | Dr. Tamer Mohamed Naguib | Medical Director | | January 12, 2025 |
| **Approved by:** | Mr. Fahad Hazam Alshammari | Hospital Director | | January 21, 2025 |