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| Detection of Lupus Inhibitors |
| **Purpose** | This procedure provides instructions for DETECTION OF LUPUS INHIBITORS, to screen for and confirm the presence of Lupus Inhibitors. Lupus Inhibitors (LA) are auto-antibodies against negatively charged phospholipids or complexes of phospholipids with either beta-2-glycoprotein 1 or clotting factors such as prothrombin. They occur in various clinical conditions, especially autoimmune diseases and are now considered to be a significant risk factor in patients with unexplained thrombosis and are often present in women who have recurrent fetal loss.The Sysmex CS-5100 is a fully automated coagulation analyzer. The CS-5100 can analyze samples using clotting, chromogenic and immunoassay methods. |
| **Policy Statements** | * This procedure applies to all clinical laboratory scientists performing coagulation tests, section supervisor and section pathologist
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| **Test Code** | LUPI, LIM |
| **Materials** | **Reagents** | **Equipment** | **Supplies** |
|  | * LA1 Screening Reagent,(10 x 2ml), PN10461887

Russell’s viper venom initiates clotting by directly activating factor X.LA antibodies prolong the LA1 Screening Reagent clotting time.The LA1 Screening Reagent is more specific for LA than PTT’s because deficiencies of the extrinsic pathway (factor VII), contact factor abnormalities (factors XI, XII) as well as hemophilic factors (factors VIII,IX) are bypassed in this reaction.* LA2 Confirmation Reagent, (10 x 1ml), PN10458687

Confirmation Reagent is similar to LA1 Screening Reagent but contains a high phospholipid concentration. The extra phospholipid counteracts the LA antibody and largely corrects the clot time.On Board Stability for LA1 and LA2 Reagents: 48hrs.It is not recommended that these products be frozen and thawed more than once after reconstitution. Reconstitute with the volume of water indicated on the vial, mix well and let sit 30 minutes before using.• Control Plasma N (BEN): PN 10446235, (10 x 1 ml)Dilute with 1ml type I deionized water. Invert gently, let stand 15 minutes before use.Stability: 16 hrs. on board analyzer• LA Control 1 (LAL) (6x1ml) PN 10872569 LA Control 2 (LAH) (6x1ml) PN 10873570 Dilute LA Controls with 1ml type I deionized water, let sit 15 minutes before use. On Board Stability for LAL and LAH Controls: 24hrs.* George King Pooled Normal Plasma (PNP): product # 0010-1, 30 x 1 mL, stored at -70°C.

Thaw in water bath or heat block for 3 minutes.Stable for 2 hours at room temperature. | * Sysmex CS-5100 System: analyzer, personal computer, printer and associated non-disposable parts.
* Reaction Tubes Sysmex CS PN 10488059.
* Plastic transfer pipettes
* 4ml sample cups

 PN 10446526* SLD Mini Cups PN 10709524
 | * TypeI deionized water, available in canisters used to collect Type I water from the Millipore system. Stable seven (7) days
* Owrens Veronal Buffer (OVB) PN10445724,

 (10 x mL )Stability: 4 days on board analyzer, 8 weeks 2-8°C* CA System Buffer PN 10873440

( 8 x 250 mL)Stability: 4 days on board analyzer, 8 weeks 2-8°C* CA Clean I

PN 10445689, (50 mL)Stability: 5 days on board analyzer,1 month 2-8°C.* CA Clean II

 PN 10708787, (45mL) ( or )• CA Clean II PN10445688  (500mL)Stability: 5 days on board analyzer2 months 5-35°C |
| **Special Safety Precautions** | BEN, LA Control low, LA Control high, and George King Pooled Normal Plasma are human source material; treat as potentially infectious. The materials from which these products have been produced were tested and found to be non-reactive for the presence of HbsAg and antibody to HIV. No known test method can offer complete assurance that the hepatitis B virus, HIV or other infectious agents are absent. Therefore all human based products should be handled with proper laboratory practices using appropriate precautions.Sodium azide present in the LA control low and high may react with lead and copper plumbing to form highly explosive metal azides. If discarded into a sink, flush with a large volume of water to prevent azide build up. |
| **Sample** | 1. Collect blood from a clean venipuncture; avoid foaming.
2. Mix nine parts of freshly collected blood with one part 3.2% (0.105 M) sodium citrate:
3. Add 1.8 mL whole blood to 0.2 mL 3.2% sodium citrate (blue-top vacutainer tube)

- or -1. Add 2.7 mL whole blood to 0.3 mL 3.2% sodium citrate (blue-top vacutainer tube)

- or -1. Special tubes must be prepared for patients whose hematocrit is > 55%. See procedure entitled *Citrate Concentration Adjustments.*
2. Invert to mix well; transport to lab at room temperature.
3. Check sample for clots with applicator sticks.
4. Centrifuge in Stat Spin for five minutes – or - 10 minutes at 3,000 rpm at room temperature.
5. Remove plasma, place in 4 mL plastic cup, centrifuge again.
6. Sample for testing: Remove plasma and place in a 4 mL plastic cup; allow for 100 μl of dead space.
7. Specimen Stability:
8. Four (4) hours when stored as plasma remaining in the capped tube above the packed cells 18 to 24°C.
9. Four (4) hours as plasma that has been separated from cells by centrifugation when stored 2 to 8°C or 18 to 24°C.
10. Two (2) weeks when stored -20°C.
11. Six (6) months when stored -70°C (rapidly frozen).
12. Plasma must be frozen if testing cannot be completed within four (4) hours.
13. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately.
14. If there is a delay in sample transport:
15. Notify supervisor or pathologist
16. If approval is given to run test, append one of the following to the result:
* “-DELA” (transport delayed)
1. Reject specimen if:
2. Clotted
3. Tube insufficiently filled (tubes may vary by no more than -10%, see comparison tubes by centrifuge).
4. Incorrect ratio of anticoagulant to blood.
5. Reject grossly hemolyzed specimens unless a new specimen cannot be drawn without causing the patient trauma or a non-hemolyzed sample is unobtainable (post-op heart, ECMO, etc.).

**If a hemolyzed sample is tested, add one of the following comments to the result depending on the amount of hemolysis:*** “-HP” (hemolysis present may affect results)
* or –
* “GRH” (gross hemolysis may interfere with testing)
1. Notify unit or physician of unacceptable specimens; enter appropriate comment in computer.
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| **Quality Control** | BEN / LA Control Low / LA Control High:BEN is pooled plasma collected from normal healthy donors, LA Low and LA High are positive control plasmas made from LA positive and normal donors.Expected values for LA1 (LP1) AND LA2 (LP2) are determined for each new lot of reagent.BEN should give a ratio between 0.9 and 1.2 with LA1Screening Reagent and LA2 Confirmation Reagent.LA Control Low should give a ratio between 1.40 and 1.70 with LA1 Screening and LA2 Confirmation Reagent.LA Control High should give a ratio between 1.7 and 2.4 with LA Screening Reagent and LA2 Confirmation Reagent. |
| **Procedure (computer)** |  Results are transmitted online in function OEM, Device code CS5M1 or CS5M2. If it is necessary to enter results manually use function MEM, Worksheet FAC, Test LUPI. For each control (C-BEN, LAL, LAH) Enter results for LA1 (LP1) and LA2 (LP2). The LA1/LA2 ratio (L12R) will be calculated in Sunquest.  |
| **Procedure** | Follow the activities in the table below to perform LUPI DETECTION OF LUPUS INHIBITOR: |
|  | **Step** | Action | **Related Document** |
|  | 1 | Load LA1 and LA2 reagent vials on CS-5100. Load the Thrombin Reagent and the Substrate Reagent in any reagent rack.Load controls into a C-Rack using SLD Mini cups.If PNP is necessary it can be poured into a 5ml vial and loaded in any reagent rack. | Training Workbook Pages 20 – 22.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
|  | 2 | To load patients, follow the procedural steps below that match the situation: |  |
|  | **If** | **Then** |  |
|  | Manual Order Processing | 1. Place rack with sample tubes on the sampler.
2. Press **Order**.
3. Enter the Rack number.
4. Select a tube position to input an order.
5. Press **Order Entry** on the Operation Panel.
6. Select **Ordinary Sample**.
7. Place the cursor in Sample No. and input the sample ID if the sample does not have a barcode. If the sample has a barcode, the 2D barcode reader can be used to input the sample ID.
8. Select the assays to be analyzed.
9. Use the down arrow to order the next sample.
10. Press **O.K**.
11. Press **Start**.
12. Confirm the sample order status on the Joblist screen.
 | Training Workbook, *page* 27.[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
|  | LIS Order Processing (Sample with barcode) | 1. Place rack with barcoded sample tube on sampler.
2. Check the host connection status. The host connection status icon must be green or orange.
3. **Press Start**.
4. After the barcodes have been read, confirm the sample order status and progress on the Joblist screen.
 | TrainingWorkbook,page 26.[Sysmex CS-5100](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)[System Training](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf)[Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
|  | Micro Mode Sampling | 1. Follow the Manual Ordering Processing steps.
2. Select the **Mc** column on the Order screen.
3. Load the un-capped tube onto the system.
4. Press **Start**.

Note: Reflex testing is not available in the Micro Mode. |  |
| **Interpretation/ Results/Critical Values** | Expected Values: LA1 <45 sec LA2 <40 sec LA1/LA2 ratio <1.40Results are expressed as a ratio of LA1/LA2 IF: LA1/LA2 > 2.0 = strong lupus inhibitor detected\* LA1/LA2 1.5-2.0 = moderate lupus inhibitor detected\* LA1/LA2 1.4-1.5 = weak lupus inhibitor detected\* LA1/LA2 < 1.4 = negative for lupus inhibitor\* \* The comment “by this testing methodology” will be appended by Sunquest to each result.If the clotting times of LA1 and LA2 are above the expected values, the results should be considered abnormal and investigated further. Results above the expected values for LA1 AND LA2 will reflexively order repeat testing on a 1+1 mix (LIM) with pooled normal plasma. These results are then reported as a ratio along with the results of the unmixed sample.The LA1/LA2 ratio may be higher than 1.4 due to a factor deficiency (factor II, V, X) or oral anticoagulant therapy. In such cases the test should be repeated using a 1+1 mixture of sample and normal plasma (see flow chart). If the abnormal result corrects to normal by the mixture, it indicates there is a factor deficiency or anticoagulant present.Results of 1.4 to 1.5 can be obtained with the 1+1 mixture when there is a strong factor deficiency. In these cases, factor determinations must be made for factors II, V, X.For further Interpretation see attached flow chart: [Flow Chart D](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200678.pdf). |
| **Dilutions** | If mixing studies are indicated load PNP and order LA1 Mix and LA2 Mix. Dilutions are made and run as indicating that they are a 1+1 mixture with George King Pooled Normal Plasma. |
| **Limitations** | 1. Samples with fibrin clots may not be used.
2. Various anticoagulants may affect results

[Effect of various anticoagulants on commonly used coagulation assays](https://starnet.childrenshc.org/References/labsop/coag/res/effect-of-various-anticoagulants-on-commonly-used-coagulation-assays.pdf)1. For comparative studies, LA1 Screening Reagent and LA2 Confirmation Reagent tests should be performed at the same time.
2. Heparin levels up to 1 unit/mL have no effect because of the presence of a neutralizing agent in both LA1 Screening Reagent and LA2 Confirmation Reagent. If heparin contamination is suspected, the heparin removal procedure should be performed.
3. Icteric, lipemic and hemolytic samples may interfere with the detection of the clot on some photoelectric instruments.
4. Mixing studies may be useful to exclude factor II, V, and X deficiencies, which may prolong both LA1 Screening Reagent and LA2 Confirmation Reagent results. Mixing studies are not indicated when the PTT is in the normal range.
5. Samples that are not spun twice to assure that they are platelet poor could result in false negative results. Phospholipid present on platelet membranes will neutralize the antibody if present and give rise to falsely normal results.
6. ISTH (International Society on Thrombosis and Hemostasis) recommends 3-step identification for lupus anticoagulants:
	1. Prolongation of a phospholipid-dependent clot assay
	2. Mixing study showing inhibitory effect
	3. Neutralization of inhibitor with a high concentration of phospholipid
 |
| **References** | 1. BCS-XP System Instruction Manual 1 000 767.0506 Manual Version 1.0 Siemens; Marburg, Germany, Copyright 2006. Automated Protocol for Dade Behring Analyzers.
2. LA1 Screening Reagent/LA2 Confirmation Reagent product insert OQGP G15 U1131.
3. Test kit Code No. OQGP (LA1)
4. Test kit Code No. OQGR (LA2)
5. Control, Lupus Anticoagulant Low, OQWE11

 1. Control, Lupus Anticoagulant High, OQWD11
2. Control Plasma N, ORKE T45 U1135, Siemens; Marburg, Germany, July 2008.
3. The Clotting Times, Vol. 4, Issue 4, Jan. 2005: Functional Laboratory Testing for Lupus Anticoagulant.[Document T - LA - APA](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200816.pdf)
4. Sysmex CS-5100 System Application Sheet RG\_39\_EN-U Rev. 2.11
5. SysmexCS-5100Training Workbook, EffectiveDate:14-Jan-2021JobAid HOOD05162003158941

[Sysmex CS-5100 System Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-5100-system-training-workbook.pdf) |
| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 9/19/22 | Initial Version, CS-5100 application |