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| **Mixing Studies of Plasma, Elevated Partial Thromboplastin Time Application**  |
| **Purpose** | This procedure provides instructions for MIXING STUDIES OF PLASMA, ELEVATED PARTIAL THROMBOPLASTIN TIME APPLICATION (EPTT), to screen for the presence of circulating anticoagulants. |
| **Principle** | This procedure is a modification of the Partial Thromboplastin Time Mixing Study (PTTM) procedure ordered by Hematology/Oncology providers as the first step in evaluating patients that present with the diagnosis of ruling out the cause of an elevated Partial Thromboplastin Time (PTT). Based on the results of the PTTM additional reflex testing will be ordered which will eliminate the need for testing that is not necessary.Mixing studies are not indicated on patients that have PTT values within the established reference range.The Sysmex CS-2500 is a fully automated coagulation analyzer. The CS-2500 can analyze samples using clotting, chromogenic and immunoassay methods. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing routine coagulation testing, section supervisor and section pathologist.
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| **Materials** | **Equipment** | **Reagents** | **Supplies** |
|  | * Sysmex CS-2500 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 | • Calcium Chloride 0.025 M:  PN 10446232. Ready for use. Stability :  • 72 hours (3 days) on  board analyzer. • Until date on label when  stored at 2-8°C, unopened. • 8 weeks when stored at 2-8°C  opened.* Dade Actin® FSL PN 10445714 10x10 mL. Purified soy phosphatides and rabbit brain phosphatides in .0001M ellagic acid with added buffer, stabilizers and preservative. A reagent for the determination of the activated partial thromboplastin time in citrated plasma.
 | * Type I deionized water, available in canisters used to collect Type I water from the Millipore system.

Stability: 7 days.* CA Clean IPN 10445689,

(50ml)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 analyzer, 2 months 5-35°C. Ready to use. |
|  |  | 1. One year’s worth of reagent is sequestered, reorder by using lot number.
2. Ready for use; mix 5-8 times, place on instrument in either cooler rack with bar code facing left.
3. Stability:

• 72 hours (3 days) on  board analyzer.* Until date on label when stored at 2 to 8°C, unopened
* Seven (7) days when stored at 2-8°C, opened.
1. If the reagent is left to stand, a green deposit may form consisting of ellagic acid and lipids, before use mix by inverting.
* George King Pooled Normal Plasma (PNP): product # 0010-1, 30 x 1 mL
* Ci-Trol Level 1**:** PN 10445731, 20 x 1ml, for the control of coagulation and fibrinolysis in the normal range.

 Ci-Trol Level 3: PN 10445733,  20 x 1 ml, for the control of coagulation and fibrinolysis in  the pathological range. |  |
| **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** | 1. Control plasmas (Ci-Trol 1, and Ci-Trol 3) should have their ranges established by each laboratory when there is a change in lot number of reagent or control material.
2. Control Plasmas (Ci-Trol 1 and Ci-Trol 3) are run:
3. At the beginning of each shift or once every eight (8) hours
4. Each time a reagent is changed.
5. Patient results cannot be reported unless control values are within expected tolerance limits.
6. If values do not fall within the expected range, test new controls then new reagents.
7. If QC is still out of range, notify the supervisor.
8. Control values are recorded daily.
9. All control values must be entered into Sunquest whether in or out of control range. Out of control values must have an appropriate modifier appended.
10. When QC data is entered, it is reviewed using Westgard rules.
* If a Westgard rule fails in Sunquest, the computer displays the result’s standard deviation from the mean.
1. If action is taken to get a control value in range, enter an appropriate comment:
* To enter corrective action in Sunquest; after the standard deviation is displayed, the prompt ENTER QC MODIFIER is displayed, use the QC modifier which best describes the action taken from the following list:

IHM - in-house maintenance; see inst logINSR - instrument recalibratedMN - mean changed, entered by Supervisor on reviewO2I3 - this control out 2 SD but in 3 SD, other controls in 2 SDOK - result ok’d by supervisor/chief techRND - repeated/new dilutionRNRG - repeated/new reagentsRNV - repeated/new vial of controlRSD - repeated/same dilutionRSVC - repeated/same vial of controlSH - short samplesSUP - excluded on supervisory reviewVENM - vendor maintenance; see inst logWRSN Westgard rule failure, supervisor notified<CR> |
| **Procedure** | Follow the activities in the table below for PERFORMING SIMPLE MIXING STUDIES OF PLASMA ( EPTT application ). |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Thaw two vials of George King Pooled Normal Plasma, run a PTT as a patient to verify reference range. After the PNP has been verified to be within the normal reference range it can then be loaded on any reagent rack in a 5ml glass vial. | Training Workbook Pages 21-23.[Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf) |
|  | 2 | Place patient sample on CS-2500; order a PTTM (PTT 1+1).If the values do not correct, there is an immediate acting circulating anticoagulant (Lupus Inhibitor) present. Freeze sample at -70° for Lupus testing. If the values correct a factor deficiency is likely but the presence of a weak heat activated inhibitor cannot be ruled out.Follow the procedure illustrated below: |  |
|  | 34 | mixing studyPatient plasma and pooled normal plasma should be incubated separately for one hour as a control for the loss of heat labile factors V and VIII during the one hour incubation period. A 1:1 mix of Patient plasma and Pooled normal plasma is incubated for one hour at the same time.After one hour the samples that were incubated separately are mixed and run on the CS-2500. An increase of 5-10 seconds would be expected due to the deterioration of Factors V and VIII during the one hour incubation period. The 1:1 mix that was incubated for one hour is also run on the CS-2500. When compared to the control any change greater than 10 seconds would likely indicate a heat dependent timed inhibitor, and would not be merely because of the loss of labile factors.Additional Notes:1. The procedure is used to differentiate a factor deficiency from an immediate acting circulating anticoagulant primarily used by HOC physicians as a quick check of hemostasis.
2. This screening procedure can be affected by the number of platelets present in the plasma, double centrifuge the sample.
3. The procedure is used to differentiate a factor deficiency from an immediate acting circulating anticoagulant primarily used by physicians as a quick check of hemostasis.
4. An immediate correction is considered to be within 3.0 seconds of the upper limit of the normal range.
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| **Reference Intervals****Result Reporting** |  | 1. Samples exhibiting gross lipemia are to be ultra-centrifuged prior to analysis.
2. Results with flags or markings are to be examined in more detail. Refer to the System Training Workbook, Sample Processing Section pages 30-38. [Sysmex CS-2500 Training Workbook](https://starnet.childrenshc.org/References/labsop/coag/res/sysmex-cs-2500-system-training-workbook.pdf)
3. Repeat patient samples with an invalid or questionable result flag.
4. Greatly prolonged results can be encountered with reagents and samples that contain air bubbles at the surface; remove all bubbles from reagents and samples.
5. Drugs that act as coagulation inhibitors may give false positive results if their presence is not detected. Performing a Thrombin time should detect the presence of the inhibitor (Heparin, Lepirudin, Bivalirudin, and Argatroban ). Samples drawn from Heparinized lines should be treated with Hepzyme®.

 In the presence of a questionable result enter with the coded comment  ANIN in Sunquest “The presence of anticoagulant inhibitor drugs such  as heparin or direct thrombin inhibitors cannot be excluded”. Reference Range: No factor deficiency or circulating anticoagulant present. [Table B – Coag Results Factor Deficiency versus Inhibitor.](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200682.pdf)See attached link for Flow Chart interpretation. [Flow Chart E: EPTT Flow Chart](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200679.pdf)Sunquest:Manual entry mode (MEM):Function: MEM <CR>Worksheet: C3 <CR>Test-1: PTM1 or AP1<CR>Test-2: <CR>CAP Method: <CR>Lots of tests appear one at a time Enter CS2S1 or CS2S2(A)ccept, (M)odify or (R)eject: A <CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>CONTR\* Enter results (xxx.x) <CR>PTM1 or AP1: Enter results (xxx.x) <CR>Enter AP2\* result: Enter results or HIDE <CR>AP2\*: Enter results or HIDE <CR>Accept (A), Modify (M), or Reject (R): A <CR> |  |
|  | • Mixing study resulting scenarios; [Document W - PTT Mixing study resulting scenarios](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/204055.pdf)• The AP1 result is the result of the immediate mix performed on the CS-2500 It should be run in column PT.1+1 or aPTT… 1+1. * The AP2 result is the result of the PTT that has been mixed and incubated together for one hour, it should be run on the CS-5100 in the column PTT…Warm. This result is entered twice, once for the calculation (enter AP2 result) and once for the result with appropriate interpretation.
* The control sample (CONTR) that is mixed after being incubated separately should be named with a unique identifier and also ordered as a test in the PTT…Warm column. This control allows for Interpretation of results that may be marginally increased after incubation due to deterioration of heat labile factors, especially factors V and VIII.

 * The control value (in seconds) and the AP2 value (in seconds) are compared. If the AP2 value is greater than 10 seconds larger than the control value is would indicate the presence of a heat dependent, timed inhibitor.
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| **References** | 1. Harmening, D.M., Clinical Hematology and Fundamentals of Hemostasis, FA Davis Company, Philadelphia, 1997, pp. 543-547 and 674-675.
2. Triplett, D.A., et al, Procedures for the Coagulation Laboratory, American Society of Clinical Pathologists Press, Chicago, 1981, pp. 69-71.
3. CLOT-ED An Educational Resource Marlies Ledford-Kraemer, Vol 3, Issue 4, Jan.2004 [Document S - All Mixed Up About Mixing Studies](http://khan.childrensmn.org/Manuals/Lab/SOP/Coag/Res/200802.pdf)
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 9/19/22 | Initial Version, CS-2500 application |