INHIBITOR / ANTICOAGULANT SCREEN
IL ACL-TOP

RC.HM.CG.PR.026.r07

Principle

An inhibitor screen is performed to determine whether a prolonged PT and/or APTT is due to a factor deficiency or an (immediate or progressive) inhibitor. A PT and/or APTT is performed on equal parts of patient and normal plasma immediately and after a 2 hour incubation at 37ºC. Significant correction immediately and remaining after two-hour incubation indicates a factor deficiency. Minimal or no correction on the initial test indicates an immediate acting inhibitor. Initial correction followed by prolongation after two-hour incubation is found with a progressive inhibitor (specific factor inhibitors such as Factor VIII inhibitor).

Specimen Collection and Handling

Refer to the Coagulation: Specimen Collection and Handling (Non –Platelet Function Tests Only) Procedure.

Supplies

EQUIPMENT:

1. See Protime / APTT procedure for IL ACL TOP
2. Non-additive Polypropylene plastic tubes (5mL)
3. Downtime labels

REAGENTS:

See Protime / APTT procedure for IL ACL TOP

1. **Dade® Hepzyme®** - Freeze-dried preparation of purified bacterial heparinase l with added stabilizers. If stored unopened at 2-8ºC, the reagent can be stored and used up to the expiration date indicated on the label.
2. **HemosIL APTT - SP** – 5 X 9 mL vials of colloidal silica dispersion with synthetic phospholipids, buffer and preservatives. Used for APTT-SP testing. Ready to use. Unopened reagent is stable until the expiration date shown on the vial when stored at 2-8ºC. Opened reagent is stable 30 days at 2-8ºC in the original vial or 5 days at 15º C on the instrument. No stirring is required. Do not freeze. **Shake silica dispersion vigorously for approximately 15 seconds or vortex for 5 seconds before use**.
3. **HemosIL Calcium Chloride**: 5 X 8 mL vials of calcium chloride (0.025 Mol/L) with preservative. Used fir APTT-SP testing. Ready to use. Unopened reagents is stable until the expiration date shown on the vial when stored at 2-8ºC. Opened reagent is stable 30 days at 2-8ºC in the original vial. For optimal stability remove reagents

from the system and store them at 2-8ºC.

4. RecombiPlasTin 2G (RTF): 5 x 20 mL vials of lyophilized recombinant human tissue factor, synthetic phospholipids with stabilizers, preservative and buffer. Allow each vial of reagent and diluent to equilibrate at 15-25˚C for at least 15 minutes before reconstitution. Pipette the exact amount required 20 mL of diluent into the vial of reagent. DO NOT POUR the contents of the diluent vial into the vial of RecombiPlasTin 2G. Replace the stopper and swirl gently. Let sit for 15 to 20 minutes at 15-25˚C and invert to mix before use. Stability after reconstitution: 10 days at 2-8°C, 5 days at 15-25°C in the original vial or 10 days at 15°C on the ACL TOP® Family. No stir bar required.

CONTROL:

1. **CryoCheck Pooled Normal Plasma (Precision Biological)** – Frozen normal, buffered human plasma. Stored at –40 to –80ºC, product is stable until the end of the month indicated on product. Thaw CryoCheck Normal Plasma at 37ºC in a water bath.

THAWING TABLE	
Aliquot Size	**37ºC Waterbath**
1.0 mL	4 minutes
1.5 mL	5 minutes
4.0 mL	5 minutes

A dry heating block is NOT recommended. Use of a timer is recommended. Bring to room temperature and invert gently to mix before use. After thawing, product may be used for 24h if capped and stored at 4ºC when not in use. Allow refrigerated plasma to acclimate to room temperature and invert gently before use. Thawed material should be discarded after 24h and should not be refrozen.

Criteria for Performing an Inhibitor Screen

PT: PT results ≤ 'x' seconds (Refer to Inhibitor Screen worksheet) do not indicate a significant increase in the PT above the normal range. Do not perform Inhibitor Screen for PT.

Initial PT results must be >'x' seconds (Refer to Inhibitor Screen worksheet) to continue with the Inhibitor Screen for the PT assay.

1. Verify that patient was not on Coumadin (warfarin) when the specimen was drawn.
   a. For valid results, patient should not have taken Coumadin for two weeks prior to blood draw. If Coumadin is present, do not continue with the Inhibitor Screen.
   b. Select Coumadin for the question "Patient on Anticoagulant". Then, select not indicated for the question "Mixing study for Abnormal PT ". "Not Indicated " will auto result in the interpretation field.

APTT:

APTT results ≤ 'x' (Refer to Inhibitor Screen worksheet) seconds do not indicate a significant increase in the APTT above the normal range. Do not perform Inhibitor Screen for APTT.

Initial APTT results must be > 'x' (Refer to Inhibitor Screen worksheet) seconds to continue with the Inhibitor Screen for the APTT assay.

1. Verify that the patient did not received heparin, LMWH or DTI at the time specimen was drawn.
    a. Confirm anti coagulation therapy or, perform a Thrombin Time test to rule out possible contamination.
        1) If the Thrombin Time is normal, perform the Inhibitor Screen.
        2) If the Thrombin Time is abnormal, treat the patient plasma with Hepzyme® and repeat the Thrombin Time. **See Hepzyme® procedure below.**
        3) If the Hepzyme® Thrombin Time corrects, do not perform the Inhibitor Screen. Add the comment "Specimen contaminated with heparin."
        4) If the Hepzyme® Thrombin Time does not correct, perform the Inhibitor Screen.

Inpatients:

Verify that the patient is not taking the following medications: argatroban*, lepirudin*, r-hirudin*, refludin*, quinidine and LMW Heparin. These medications can affect both the PT and APTT. For valid results, patients should not have received direct thrombin inhibitors for 48 hours.

Hepzyme® Procedure:

1. Add 1mL platelet-poor citrated plasma to Dade® Hepzyme® vial, re-close with a rubber stopper, and invert gently 5 to 10 times.
2. Allow vial to stand at room temperature (15-25 ºC) for 15 minutes.
3. Perform the Thrombin Time test.

NOTE: An enhanced clean for the sample probe must be performed on the ACL-TOP after running a Hepzyme® treated sample.

Procedure

1. Perform a PT and APTT on patient's undiluted citrated plasma to obtain the initial results and to determine if it is necessary to perform the Inhibitor Screen (see Criteria for Performing an Inhibitor Screen above). If it is necessary to continue with the Inhibitor Screen then Select Yes for the question "Mixing Study for Abnormal (PT or PTT)", continue with the following steps:
2. **1:1 Immediate:** Prepare the following specimens in 5 mL non-additive polypropylene tubes:
   Tube #1: Add 1.0 mL patient's plasma + 1.0 mL Cryo Check Pooled Normal Plasma (1:1 dilution) {if sufficient patient plasma}.
   Tube #2: Add 1.5 mL CryoCheck Pooled Normal Plasma, undiluted {control}
3. Add a downtime label to both tube #1 and tube #2. Order the test(s) required (PT and/or APTT) on the ACL-TOP.
4. Remove caps from 5 mL tubes and immediately perform a PT and/or APTT on both tubes.
5. If the APTT 1:1 immediate result is 'x- y' sec (Refer to Inhibitor Screen worksheet) , repeat the initial APTT, tube #1 {1:1 immediate} and tube #2 {control/PNP immediate} using HemosIL APTT-SP test. It is not used in the incubation step below.
6. **1:1 Post 2 hour Incubation:** Incubate tube #1 and tube #2 in 37ºC water bath for two hours.

7. After incubation, re-order the test(s) {PT and/or APTT} on the ACL-Top worklist and run the sample(s) immediately. For best results, use the same instrument and the same reagents.
8. Refer to Inhibitor Screen worksheet for directive on when to perform a Platelet Neutralization procedure.
9. Use Softlab LIS template for "Test" (INACS) to enter results. Follow the prompt. "Save and verify results". Document all test results on the Inhibitor Screen Worksheet. Place worksheet in the designated pathologist folder for interpretation.

Expected Values

INTERPRETATION:

Refer to Inhibitor Screen Worksheet for interpretations and further instructions.

NORMAL VALUES:

No inhibitor detected.

RESULTING IN THE LIS:

1. All inhibitor screens that have been set up for a mixing study must be referred to the pathologist for interpretation.

TAT:

Within 4h after specimen collection

References

1. Sirridge, MS: Laboratory Evaluation of Hemostasis. 2nd edition, 1974, Lea and Febiger, pp. 184-186.
2. Third International Survey on Lupus Anticoagulants, Ball Memorial Hospital, Muncie, IN, 1994.
3. Dade® Hepzyme®, package insert, Dade Behring, Marburg, Germany, August 2004

Authorized Reviewers

Medical Director, Coagulation

INHIBITOR / ANTICOAGULANT SCREEN – IL ACL-TOP

Document Control

Location of Master: Coagulation Procedure Manual
Master electronic file stored on the Beaumont Laboratory server:
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Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Jon B. Goller, MT(ASCP)	11/2001			
Approved by: Joan C. Mattson, MD	12/05/2001			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Joan C. Mattson, MD	12/05/2001		New procedure, reagents, instrument. Updated to add 4:1 dilutions.	
Noelle Procopio, MT(ASCP)SH	12/30/2002		Pg. 3 email note added.	
Joan C. Mattson, MD	02/06/2003		Deleted 4:1 dilution because uninformative.	
Joan C. Mattson, MD	03/26/2003		PNP added to algorithm.	
Joan C. Mattson, MD	12/27/2004		Changed one hour incubation to two hours.	
Joan C. Mattson, MD	01/18/2006	00	Standardized procedure format.	
Marc Smith, MD	04/20/2007	01	Added Hepzyme procedure.	
Marc Smith, MD	01/05/2009	02	Referred specimen handling to the Coagulation: Specimen Collection and Handling procedure; updated from CA1500 to CA7000; deleted algorithm; referred interpretation to worksheet; added LMW Heparin and direct thrombin inhibitors to Criteria for performing inhibitor screen; updated principle; updated control info; referred all inhibitor screens to pathologist for interpretation.	

Marc Smith, MD	06/16/2009	03	Updated criteria for performing inhibitor screen; referred to inhibitor screen worksheet; updated resulting in the LIS; updated test name.	
Marc Smith, MD	08/06/2010		No change	
Marc Smith, MD	07/14/2011	04	Updated criteria for performing the inhibitor screen and thrombin time test. RC.HM added to SOP#; new format	
Marc Smith, MD	07/01/2013	05	Pg,2 Updated criteria for performing inhibitor screen for PT.	OK
Marc Smith, MD	04/02/2015		No change	OK
Marc Smith, MD	04/05/2017	06	Pg. 2 added instruction for result LIS documentation	OK
Elizabeth Sykes, MD	02/22/2018			
Marc Smith, MD	10/02/2018	**07**	Updated from CA7000 to IL ACL-TOP. Changed reagents used.	OK
Peter Millward, MD	09/27/2018			