Beaumont Laboratory
Royal Oak

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Related Documents:
RC.CG.PR.001 Coagulation Tests: Specimen Collection and Handling (Non-Platelet Function Tests Only)
RC.HM.CG.PR.002 Coagulation Tests: Reportable Limits and Normal / Therapeutic Values
RC.HM.CG.PR.007 Coagulation Correlations
RC.HM.CG.PR.095 IL ACL TOP Operations Procedure

PLATELET NEUTRALIZATION PROCEDURE (PNP) IL ACL TOP®

RC.HM.CG.PR.054.r08

Principle

The platelet neutralization procedure (PNP) is based on the ability of platelets to correct significantly in vitro coagulation abnormalities. The disrupted platelet membranes present in the freeze-thawed platelet suspension neutralize phospholipid antibodies present in the plasma of patients with lupus anticoagulant. After the patient plasma is mixed with the freeze-thawed platelet suspension, the aPTT will be "corrected" when compared with the original baseline aPTT.

Specimen Collection and Handling

Refer to Coagulation Tests: Specimen Collection and Handling (Non-Platelet Function Tests Only) procedure

Supplies

EQUIPMENT

a. IL Coagulation Analyzer
b. Cuvette
c. IL Reagent racks and sample racks
d. Cleaning and Rinse solutions
e. Serological and automatic pipettes

REAGENTS

A. Precision Biologic, CryoCheck Platelet Lysate – When stored at –70ºC, Platelet Lysate is stable to expiration date indicated on the product packaging. Thaw each vial at 37^0C in a waterbath for 4 minutes. It may be used for up to 8 hours after thawing if capped in the original vial and held at 2 to 8ºC. Allow refrigerated plasma to acclimate to room temperature (20 to 25ºC) and invert gently prior to use. Thawed material should be discarded after 8h and should not be refrozen.

B. HemosIL APTT - SP – 5 X 9 mL vials of colloidal silica dispersion with synthetic phospholipids, buffer and preservatives. Used for aPTT 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.

C. HemosIL Calcium Chloride: 5 X 8 mL vials of calcium chloride (0.025 Mol/L) with preservative. Used for 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.

D. Factor Diluent Buffer

CONTROLS

A. HemosIL **Normal Control 1**: 10 x1 mL vials of lyophilized human plasma containing buffer, stabilizers and preservatives. Dissolve the contents of each vial with 1mL of DI water or equivalent. Replace the stopper and swirl gently. Complete reconstitution of the product is required. Keep the control at 15-25°C for 30 minutes and invert to mix before use. Do not shake to avoid foam formation. Control is stable after reconstitution for 24 hours at 2-8°C or 15-25°C onboard the ACL TOP® Family.

B. Precision Biologic, CryoCheck Lupus Positive Control - When stored at –70^{0}C, control is stable to the expiry date indicated on the product packaging. Thaw each vial in a 37ºC waterbath for 4 minutes. After thawing, it is stable for 8h if kept at 2-8°C.and in the original vial. Allow refrigerated plasma to acclimate to room temperature (20 to 25ºC) and invert gently prior to use.

C. Precision Biologic, CryoCheck Weak Lupus Positive Control - When stored at –70°C, control is stable to the expiry date indicated on the product packaging. Thaw each vial in a 37ºC waterbath for 4 minutes. After thawing, it is stable for 8h if kept at 2 to 8ºC and in the original vial. Allow refrigerated plasma to acclimate to room temperature (20 to 25ºC) and invert gently prior to use.

Quality Control

A. Quality control consists of HemosIL Normal Control 1 and CryoCheck Lupus Weak Positive Control and CryoCheck Lupus Positive Control.

B. Frequency of CONTROL Use:

1. HemosIL Normal Control 1, CryoCheck Weak Lupus Positive Control and CryoCheck Lupus Positive Control must be run as part of each PNP run.

2. The PNP assay on IL ACL TOP® is also monitored through the use of CAP proficiency surveys or equivalent.

Procedure

A. Refer to IL Coagulation Operation Procedure.

Expected Values

A. Any unreasonable result is to be repeated.
B. Enter PNP buffer and platelet results from IL ACL TOP.
C. Using the formula:
    1. PNP Result = PNP Buffer – PNP Platelets
    2. A correction of ≥**4.0** seconds indicates a positive result

3. A correction of <**4.0** seconds indicates a negative result

D. In the "Platelet Neutralization" field of the Inhibitor Screen, report out the test as Positive or Negative. The pathologist comment is added to the interpretation field.

NORMAL RANGE
A. Negative

REPORTABLE RANGE
A. Positive or Negative

TAT
Routine PNP – 24 hours (Monday – Friday)

Limitations

A. Bubbles interfere with Siemens IL ACL TOP liquid level sensor and therefore sampling. Make sure there are not any bubbles in any sample or reagent.
B. Platelet Lysate contains platelet factor 4 which neutralizes the heparin indicating corrections of the sample. Heparinized samples must not be used. Samples with coumadin do not affect results.

References

A. ACL TOP Online Operator's Manual, Version 2.2, June 2017
B. Precision Biologic Platelet Lysate Reagent package insert, Dartmouth, NS, 2001
C. HemosIL Normal 1 Control package insert, September 2016
D. *National Committee for Clinical Laboratory Standards: Collection, Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays*. Approved Guideline, 2nd Edition. NCCLS Publication H221-A2. Wayne, PA, 1991.
E. *National Committee for Clinical Laboratory Standards: One-Stage Prothrombin Test (PT) and Activated Partial Thromboplastin Time Test (APTT)*. Approved Guideline. NCCLS Publication H47-A. Wayne, PA, 1995.
F. HemosIL APTT-SP, package insert, Lexington MA, 02/2005.

Authorized Reviewers
Medical Director, Coagulation

PLATELET NEUTRALIZATION PROCEDURE (PNP) – SIEMENS BCS®

Document Control

Location of Master: Coagulation Procedure Manual
Master electronic file stored on the Beaumont Laboratory server:
S:\ HEMACOAG\Document Control\Coagulation\Procedure\Master Documents\Platelet neutralization procedure.doc
Number of Controlled Copies posted for educational purposes: 0
Number of circulating Controlled Copies: 0
Location of circulating Controlled Copies: NA

Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Maria Luhring, MT(ASCP)	03/2003			
Approved by: Joan C. Mattson, MD	03/26/2003			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Joan C. Mattson, MD	03/26/2003		New protocol for commercial procedure	
Joan C. Mattson, MD	01/09/2004		No change	
Noelle Procopio, MT(ASCP)SH	01/04/2005		No change	
Joan C. Mattson, MD	09/06/2005	00	Standardized procedure format, expanded on reagents and controls, pg.2.	
Noelle Procopio, MT(ASCP)SH	12/22/2006		No change	
Marc Smith, MD	05/25/2007	01	Pg. 2 added HemosIL-SP reagent, deleted Actin FSL reagent. Pg. 5 change reference range to $\geq$ 3.5 seconds-positive $\leq$ 3.5 negative pg. 5 deleted E-add 2PTT charge for PNP test.	
Marc Smith, MD	08/02/2007	02	Pg. 1 changed 4°C to room temperature 20-25°C. Added Stat Spin Centrifuge. Pg. 2 changed packet to bottle. Pg. 7 delete B resulting is performed under Misc Coag.	

Marc Smith, MD	12/23/2008	03	Referred specimen collection to separate procedure; referred procedure steps to BCS Operations procedure. Edited expected values PNP procedure. Updated weak lupus positive and lupus positive control; updated where reportable field is located in LIS; updated references.	
Marc Smith, MD	08/31/2009		No Change	
Marc Smith, MD	04/16/2010	04	Deleted Control N and added Precision Biologic Pooled Normal Plasma.	
Marc Smith, MD	07/13/2011		RC.HM added to SOP#; new format	
Marc Smith, MD	07/10/2013	05	Pg.3 changed >3.5 seconds to >5.0 seconds (Positive) and <3.5 seconds to <5.0 seconds (Negative).Pg 1. Deleted Precision Biologic and added Control N; updated Dade to Siemens.	OK
Marc Smith, MD	03/05/2014	06	Pg 2 deleted cryo normal plasma and added Control N.	OK
Marc Smith, MD	04/01/2016	**07**	Added BCSXP operations procedure to document.	OK
Elizabeth Sykes, MD	02/22/2018			
Marc Smith, MD	04/16/2018		Updated logo only	OK
Peter Millward, MD	3/13/2019			
Marc Smith, MD	6/16/2020	08	Changed Reagents and controls Pg 2. Expected Value Pg 3 New Intstument	OK