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Platelet Neutralization (PNP)-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document describes how to preform platelet neutralizing procedure. 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 Activated Partial Thromboplastin Time aPTT.

II. ACRONYMS:

A. Activated Partial Thromboplastin Time (aPTT)
B. Instrumentation Laboratory (IL)
C. Positive Lupus (PL)
D. Quality Control (QC)
E. Weak Positive Lupus (WLP)

III. SPECIMEN COLLECTION AND HANDLING:

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

IV. SUPLIES/EQUIPMENTS:

A. IL Coagulation Analyzer
B. Cuvette
C. IL Reagent racks and sample racks
D. Cleaning and Rinse solutions
E. Serological and automatic pipettes

V. REAGENTS:

A. **Precision Biologic, CryoCheck Platelet Lysate** – When stored at –70^{o}C, Platelet Lysate is stable to expiration date indicated on the product packaging. Thaw each vial at 37^{0}C 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 8^{o}C. Allow refrigerated plasma to acclimate to room temperature (20 to 25^{o}C) and invert gently prior to use. Thawed material should be discarded after 8h and should not be refrozen.

B. **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 2-8^{o}C. Opened reagent is stable 30 days at 2-8^{o}C in the original vial or 5 days at 15^{o} 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 2-8^{o}C. Opened reagent is stable 30 days at 2-8^{o}C in the original vial. For optimal stability remove reagents from the system and store them at 2-8^{o}C.

D. **Factor Diluent Buffer-** Ready to use.

VI. 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 on board 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 37^{o}C 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 25^{o}C) and invert gently prior to use.

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

VII. 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.

VIII. PROCEDURE:

A. Verify the lot number of reagent and QC on IL ACL TOP and record the numbers in the log.

B. Gently invert vials of reagents and QC.

C. Check for bubbles before loading reagents and controls on IL ACL TOP.

D. Load **APTT-CaCL2 in R3-R6 lane.**

E. Load **APTT-SP in D3, R1-R4.**

F. Load **PNP-FD in 10 mL glass vial in D1 or D2 and assign the reagent.**

G. Load **PNP-Lys** in 4 mL glass vial in **D1 or D2** and assign the reagent

H. Load **PL and WPL** in 2 mL sample cups in **S1-S12** and assign the quality controls.

I. Verify that all levels of QC are withing acceptable range before running patients.

J. Document any troubleshooting performed if QC fails.

K. Thaw patients samples for 5- 10 minutes in water bath. It is unacceptable to run samples that are thawed at RT

L. Refer to IL Operations Procedure for any instrumentation details.

IX. 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: PNP Result = PNP Buffer – PNP Platelets

1. A correction of >4.0 seconds indicates a positive result.
2. 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.

X. NORMAL RANGE:

Negative

XI. REPORTABLE RANGE:

Positive or Negative

XII. TURNAROUND TIME:

Routine PNP – 24 hours (Monday – Friday)

XIII. 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.

XIV. 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.

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
CP Chief Medical Director	Peter Millward: Chief, Pathology Service Line	5/13/2021
Coagulation Medical Director Designee	Marc Smith: System Med Dir, Coagulation	5/13/2021
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Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	5/11/2021
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Applicability

Royal Oak