PrecisionBioLogic

CRYO*check*™ IVD

PLATELET NEUTRALIZATION PROCEDURE REAGENT

PLATELET LYSATE

Intended Use

CRYO*check* Platelet Lysate is intended for use in the Platelet Neutralization Procedure (PNP) which is useful in detecting the presence of lupus anticoagulants (LA) in citrated human plasma.

Summary and Principle

The activated partial thromboplastin time (APTT) is an established screening assay used to assess the intrinsic coagulation pathway. Abnormal prolongation of the APTT has many etiologies, one of the more common of which is the presence of LA[1,2]. LA are immunoglobulins which are directed against a variety of anionic phospholipids and/or phospholipid-protein complexes and consequently interfere with in vitro phospholipid dependent coagulation tests[3,4].

In 1983, Triplett *et al.* introduced the PNP based on the demonstrated principle that the products of platelet lysis neutralized the inhibitory effect of LA[5]. The PNP offered the further advantage of being able to differentiate between LA and specific factor inhibitors. Subsequently, the SSC Subcommittee on Lupus Anticoagulants established four specific criteria to confirm the presence of LA, including the relative correction of the LA defect by the addition of washed, frozen-thawed platelets, phosphatidylserine or hexagonal phase phospholipids[6]. In a follow up study, the SSC Subcommittee on Lupus Anticoagulants recommended that for samples suspected of having LA, at least one additional screening assay must be performed if the initial assay is negative[6].

Reagents

CRYO*check* Platelet Lysate is prepared from human platelets derived from normal healthy donors. The platelet concentrate is adjusted to be equivalent to 250,000 - 300,000 platelets/µL.

All blood products should be treated as potentially infectious. Source material from which this product was derived was found to be negative when tested in accordance with current required tests for transfusion-transmitted diseases. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents. Accordingly, these human blood-based products should be handled and discarded as recommended for any potentially infectious human specimen[7].

Storage, Preparation and Handling

When stored at -40 to -80 °C, CRYO*check* Platelet Lysate is stable to the end of the month indicated on the product packaging.

Thaw each vial at 37°C (± 1°C) in a waterbath. **The use of a dry bath or heating block for thawing is not recommended.** Thaw times are important and should be strictly adhered to. The use of a timer is recommended. Refer to the Thawing Table for recommended thawing times based on aliquot size. Allow thawed lysate to acclimate to room temperature (18 to 25 °C) and invert gently prior to use.

Thawing Table	
Aliquot Size	**37 °C (± 1 °C) Waterbath**
1.0 mL	4 minutes

CRYO*check* Platelet Lysate may be used for up to eight hours after thawing, if capped in the original vial and maintained at 2 to 8 °C. Allow refrigerated lysate to acclimate to room temperature (18 to 25 °C) and invert gently prior to use. **Thawed material should be discarded after eight hours and should not be refrozen.**

Availability

Product	Catalog #	Format
CRYO*check* Platelet Lysate	PNP-10	25 vials x 1.0 mL

Instruments

Each lab should prepare the local instrument in accordance with the manufacturer's instructions for use.

Procedure

Materials Provided

- CRYO*check* Platelet Lysate

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (± 1 °C)
- Assay reagents
- Tris buffer saline 0.05 M; pH 7.5
- Coagulation instrument or assay system
- Quality control material (e.g. CRYO*check* Lupus Positive control, CRYO*check* Weak Lupus Positive control)
- Sample Cups
- Plastic disposable pipettes
- Volumetric pipette
- Timer

Specimen Collection and Preparation

Patient samples should be collected into 105 - 109 mmol/L sodium citrate dihydrate anticoagulant (3.2%) in a ratio of 9 parts blood to 1 part anticoagulant. Patient plasma is derived by centrifugation at 1500 x g for 15 minutes in order to achieve platelet-poor plasma (<10,000 platelets/µL) and should be tested within four hours of collection when maintained at 2 to 4 °C in accordance with CLSI guidelines[8]. If samples are to be frozen before testing, plasmas should be centrifuged a second time, and stored at -20 °C or below.

Establishing a Baseline APTT

1. Prepare APTT reagent, $CaCl_2$, and quality control materials according to manufacturer's directions.
2. Pre-warm APTT reagent and $CaCl_2$ to 37 °C (±1 °C).
3. Pipette 0.1 mL of normal control plasma into a reaction cuvette.
4. Add 0.1 mL of pre-warmed APTT reagent, mix, and incubate at 37 °C (±1 °C) according to manufacturer's directions.
5. Add 0.1 mL of pre-warmed $CaCl_2$ and simultaneously initiate clot timer. Record clotting time in seconds.
6. Repeat steps 3 to 5 for each of the test plasmas and quality control materials.

Platelet Neutralization Procedure – Saline Dilution

1. Prepare APTT reagent, $CaCl_2$, quality control materials, and buffered saline according to manufacturer's directions.
2. Pre-warm APTT reagent and $CaCl_2$ to 37 °C (±1 °C).
3. Pipette 0.1 mL of normal control plasma into a test cuvette.
4. Add 0.1 mL of Tris buffered saline.
5. Add 0.1 mL of pre-warmed APTT reagent, mix and incubate at 37 °C (±1 °C) according to manufacturer's directions.
6. Add 0.1 mL of pre-warmed $CaCl_2$ and simultaneously initiate clot timer.
7. Record clotting time in seconds of the Saline Dilution.
8. Repeat steps 3 to 7 for each test plasma and quality control material.

Platelet Neutralization Procedure – Platelet Lysate Dilution

1. Prepare APTT reagent, $CaCl_2$, quality control materials, and CRYO*check* Platelet Lysate according to manufacturer's directions.
2. Pre-warm APTT reagent and $CaCl_2$ to 37 °C (±1 °C).
3. Pipette 0.1 mL of normal control plasma into a test cuvette.
4. Add 0.1 mL of CRYO*check* Platelet Lysate.
5. Add 0.1 mL of pre-warmed APTT reagent, mix and incubate at 37 °C (±1 °C) according to manufacturer's directions.
6. Add 0.1 mL of pre-warmed $CaCl_2$ and simultaneously initiate clot timer.
7. Record clotting time in seconds of the Platelet Dilution.
8. Repeat steps 3 to 7 for each test plasma and quality control material.

Results

For each test plasma, the clotting time of the Saline Dilution is compared to the clotting time of the Platelet Lysate Dilution. The difference between the two results is then compared to the normal cutoff value, as discussed in Expected Values, and interpreted as either LA positive or LA negative based on the degree of reduction in the clotting time. In the presence of LA, the clotting time of the Saline Dilution should be shorter than, or equal to, the clotting time of the Baseline APTT.

PNP results alone cannot be used to conclusively diagnose the presence of LA[6]

Quality Control

Each laboratory should establish its own quality control (QC) range**s** using acceptable statistical methods. These QC ranges may then be used to monitor and validate the integrity of the test system[9]. For all coagulation tests, the laboratory must include at least two levels of control for every eight hours of operation and any time a change in reagents occurs[10].

Commercial lyophilized quality control plasmas containing unspecified levels of citrate and platelets are not recommended as they may give erroneous results[11,12].

Limitations of the Procedure

When proper control values are not obtained, assessment of each component of the test system including reagents, control plasmas, instrumentation and operator technique must be undertaken in order to ascertain that all other components are functioning properly. Patient samples containing heparin, specific factor inhibitors, or oral anticoagulants may exhibit false positive results[5,6].

Expected Values

Due to the varied phospholipid content and diverse sensitivity of commercial APTT reagents to LA[13,14], as well as differences in instrument clot detection and methodology[15], results may vary from laboratory to laboratory. Studies were performed on 25 normal individuals using photo-optical and mechanical clot detection instrumentation. Photo-optical instrumentation generated a mean neutralization of 2.1 seconds with a standard deviation of 1.1. Mechanical clot detection instrumentation generated a mean neutralization of 3.7 seconds with a standard deviation of 1.2. Each laboratory should establish its own parameters for expected values (i.e. normal cutoff value) by evaluating a representative sample of known normal and LA positive patient samples. In accordance with SSC guidelines, PNP clotting time corrections greater than three standard deviations above the normal population are considered to be LA positive[6].

The following PNP test results were generated on five known normal patients and 10 known positive LA patients using organon Teknika Auto APTT reagent with CRYO*check* Platelet Lysate on a ST4 analyzer.

Patient Sample	Baseline APTT (sec)	Saline Dilution (sec)	Platelet Lysate Dilution (sec)	Correction (sec)
Normal 1	31.4	35.8	36.4	(0.6)
Normal 2	28.7	30.4	31.3	(0.9)
Normal 3	32.6	32.3	32.2	0.1
Normal 4	30.5	35.4	34.5	0.9
Normal 5	31.9	34.7	33.7	1.0
LA 1	75.6	63.1	41.0	22.1
LA 2	55.8	43.6	37.5	6.1
LA 3	51.7	40.9	36.4	4.5
LA 4	103.8	89.6	45.1	44.5
LA 5	62.5	49.4	42.1	7.3
LA 6	53.7	47.0	40.4	6.6
LA 7	47.1	41.2	37.8	3.4
LA 8	80.5	70.0	41.1	28.9
LA 9	64.7	57.7	38.2	19.5
LA 10	73.3	59.2	44.9	14.3

Performance Characteristics

A R^2=0.975 was recovered in a correlation study with an established method using normal and LA positive patient samples. In precision studies over eight hours using two lots of CRYO*check* Platelet Lysate with two lots of CRYO*check* Lupus Positive control, the following precision data was recovered:

Platelet Lysate Lot #	Lupus Positive Control Lot #	Mean PNP Ratio	SD
PL01	6130	1.94	0.385
PL01	6120	2.10	0.286
PE001	6130	1.94	0.278
PE001	6120	2.14	0.377

Bibliography

1. Corrigan JJ. Coagulation inhibitors. Am J Ped Hem/Oncol 1980; 2(3):281-288.
2. Feinstein DI, Rapaport SI. Acquired inhibitors of blood coagulation. Progress in Hemostasis and Thrombosis 1972; 1:75-95.
3. Thiagarajan P, Shapiro SS, DeMarco L. Monoclonal immunoglobulin M lambda coagulation inhibitor with phospholipid specificity. Mechanism of a Lupus Anticoagulant. J Clin Invest 1980; 66(3):397-405.
4. Bevers EM, Galli M, Barbui T, Comfurious P, Zwool RFA. Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. Thromb Haemost 1991; 66(6):629-632.
5. Triplett DA, Brandt JT, Kaczor D, Schaeffer J. Laboratory diagnosis of lupus inhibitors. A comparison of the tissue thromboplastin inhibition procedure with a new platelet neutralization procedure. Am J Clin Pathol 1983; 79(6):678-682.
6. Pengo V, Tripodi A, Reber G, Rand JH, ortel TL, Galli M, De Groot PG; Update of the guidelines for lupus anticoagulant detection. J Thromb Haemost 2009; 7(10):1737-1740.
7. Biosafety in Microbiological and Biomedical Laboratories 6th ed. centers for Disease control and Prevention / National Institutes of Health, 2020.
8. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based coagulation Assays and Molecular Hemostasis Assays; Approved Guideline- Fifth Edition, CLSI, H21-A5; Vol. 28, No. 5, 2008.
9. Cembrowski GS, Carey RN. Laboratory quality management. Chicago: ASCP Press; 1989. p. 166-171.
10. CLIA 2004 – code of Federal Regulations, 42CFR493.1269, 2004.
11. Adcock DM, Kressin Dc, Marlar RA. Effect of 3.2% vs. 3.8% sodium citrate concentration on routine coagulation testing. Am J Clin Pathol 1997; 107(1):105-110.
12. Hirst CF, Poller L. The cause of turbidity in lyophilized plasmas and its effects on coagulation tests. J Clin Pathol 1992; 45(8):701-703.
13. Mannucci PM, Canciani MT, Mari D, Meucci P. The varied sensitivity of partial thromboplastin and prothrombin time reagents in the demonstration of the lupus-like anticoagulant. Scand J Haematol 1979; 22:423-432.
14. Adcock DM, Marlar RA. Activated partial thromboplastin time reagent sensitivity to the presence of lupus anticoagulants. Arch Pathol Lab Med 1992; 116:837-840.
15. Gilmer PR. Preanalytical variables in coagulation testing. In: Triplett DA, editor. Laboratory evaluation of coagulation. Illinois: ASCP Press; 1982. p. 1-8.

Symbols Used

IVD	In vitro diagnostic medical device		Biological risks
LOT	Batch code		Manufacturer
REF	Catalogue number	EC REP	Authorized representative in the European Community / European Union
	Use by date	**Rx ONLY**	For prescription use only
	Temperature limit		Consult electronic instructions for use

EC REP European Authorized Representative (Regulatory affairs only)
Emergo Europe—Westervoortsedijk 60, 6827 AT Arnhem, The Netherlands

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