



CRYOcheck™

Platelet Neutralization Procedure Reagent PLATELET LYSATE

Intended Use

CRYOcheck 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 LA⁵. 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 phospholipids⁶. In a followup 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 negative⁶.

Reagents

CRYOcheck 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/ μ L.



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 FDA required tests. 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 specimen⁷.

Storage and Handling

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

Thaw each vial at 37 °C (\pm 1 °C) in a waterbath. **The use of a dry bath or heating block for thawing is not recommended.** Thawing 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 (\pm 1 °C) Waterbath
1.0 mL	4 minutes

CRYOcheck 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
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

- CRYOcheck Platelet Lysate

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (\pm 1 °C)
- Assay reagents
- Tris buffered saline 0.05 M; pH 7.5
- Coagulation instrument or assay system
- Quality control material (e.g. CRYOcheck Lupus Positive Control, CRYOcheck 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/ μ L) and should be tested within four hours of collection when maintained at 2 to 4 °C in accordance with CLSI guidelines⁸. 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, CaCl₂, and quality control materials according to manufacturer's directions.
2. Pre-warm APTT reagent and CaCl₂ to 37 °C (\pm 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 (\pm 1 °C) according to manufacturer's directions.
5. Add 0.1 mL of pre-warmed CaCl₂ 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, CaCl₂, quality control materials, and buffered saline according to manufacturer's directions.
2. Pre-warm APTT reagent and CaCl₂ to 37 °C (\pm 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 (\pm 1 °C) according to manufacturer's directions.
6. Add 0.1 mL of pre-warmed CaCl₂ 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, CaCl₂, quality control materials, and platelet lysate according to manufacturer's directions.
2. Pre-warm APTT reagent and CaCl₂ to 37 °C (\pm 1 °C).
3. Pipette 0.1 mL of normal control plasma into a reaction cuvette.
4. Add 0.1 mL of CRYOcheck Platelet Lysate.
5. Add 0.1 mL of pre-warmed APTT reagent, mix and incubate at 37 °C (\pm 1 °C) according to manufacturer's directions.
6. Add 0.1 mL of pre-warmed CaCl₂ and simultaneously initiate clot timer.
7. Record clotting time in seconds of the Platelet Lysate Dilution.
8. Repeat steps 3 to 7 for each test plasma and quality control material.

Quality Control

Each laboratory should establish its own quality control (QC) ranges using acceptable statistical methods. These QC ranges may then be used to monitor and validate the integrity of the test system⁹. 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 occurs¹⁰.

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

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 LA⁶.

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 methodology¹⁵, 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 positive⁶.

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 CRYOcheck 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 R²=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 CRYOcheck Platelet Lysate with two lots of CRYOcheck 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

