



THROMBOPLASTIN-DS

I. Intended Use

Pacific Hemostasis® Thromboplastin-DS is intended for use in performing the one-stage prothrombin time (PT) test and PT-based factor assays.

II. Summary and Principles

The PT is used as a screening tool and as a quantitative test for coagulation factors in the extrinsic and common pathways. This test will be prolonged in patients with acquired or congenital disorders that reduce the activity of factors I (fibrinogen), II (Prothrombin), V, VII, and X. The PT is also widely used to monitor oral anticoagulant therapy.^{1,2} Oral anticoagulants reduce the activity of vitamin-K dependent clotting factors (II, VII, IX, X, Protein C, and Protein S), and the PT is prolonged as a result.

The one-stage PT measures the clotting time of plasma after adding a source of tissue factor (thromboplastin) and calcium. The recalcification of plasma in the presence of tissue factor generates activated Factor Xa (F.Xa). F.Xa in turn activates Prothrombin to thrombin, which converts fibrinogen to an insoluble fibrin clot.

III. Reagent

For *in vitro* diagnostic use.

Composition: <2% rabbit brain tissue, 0.013% sodium azide, 5% buffers, salts, and stabilizers

Store unopened vials at 2-8°C. Reconstitute with preservative-free distilled/deionized water according to the Thromboplastin-DS vial label, swirl gently and let the vial stand undisturbed for 15 minutes at room temperature. Do not invert the vial or mix vigorously. Reconstitution fluid is available if the quality of water is questionable. After reconstitution, the reagent when stored stoppered is stable for 7 days at 2-8°C, 8 hours at 37°C, and for 24 hours at room temperature (15-25°C).⁸ Store at 2-8°C when not in use. **Do not freeze.**

Mix gently before each use. Provide some mechanism, such as a magnetic stirrer, to maintain adequate suspension during use. Lack of vacuum in vials, erratic results, quality control values outside established ranges, or product color variations could indicate deterioration. However, poor performance could also be due to other factors within the test system.

Warning: Thromboplastin-DS contains sodium azide. Sodium azide under acidic conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water before being discarded. Upon disposal, azide compounds should be flushed with large volumes of water. These precautions are recommended to avoid deposits in metal pipes in which explosive conditions may develop.

IV. Specimen Collection

3.2% (0.105M) trisodium citrate anticoagulant is recommended for coagulation assays. Avoid hemolysis and contamination by tissue fluids. Samples that have less than 90% of the expected fill volume should be rejected. Centrifuge blood for 15 minutes at 1500 x g. Test within 2 hours if samples are held at 22-24°C. If testing is not completed within 24 hours, plasma should be frozen at -20°C for up to two weeks or -70°C for up to 6 months. For more details on specimen collection and storage, see NCCLS Document H21-A4.³

- Do not delay mixing the blood with anticoagulant.
- Avoid foaming the specimen.

- Use only plastic or siliconized glass containers.
- Turbid, icteric, lipemic, or hemolyzed specimens may generate erroneous results.
- Freezing and thawing plasma that contains residual cells will generate damaged cell membranes that can affect results.
- Acute inflammatory reactions can shorten PT results because of elevated fibrinogen.
- Plasma samples with hematocrits outside the range of 20-55% may be improperly anticoagulated and should be adjusted appropriately.

V. Test Procedure

Materials Provided: Thromboplastin-DS Reagent, 10 x 4 mL, 10 x10 mL, or 10 x 2 mL

Materials Required, But Not Provided:

Distilled or Deionized water, or Pacific Hemostasis Reconstitution Fluid

Stopwatch or timer

Precision pipette: 0.1 and 0.2 mL

Normal and abnormal controls such as Pacific Hemostasis Coagulation Control Plasmas, Level 1, 2, and 3

Thromboplastin-DS is suitable for use with manual, mechanical, photo-optical, nephelometric or other means of clot detection. Follow manufacturer's recommendations for proper use of instrumentation. For manual assays:

- A. Prewarm Thromboplastin-DS to 37°C.
- B. Add 0.1 mL test plasma to cuvette and prewarm to 37°C.
- C. Forcibly add 0.2 mL warmed Thromboplastin-DS to the test plasma and time clot formation.

VI. Quality Control

Normal and abnormal plasmas such as Pacific Hemostasis Coagulation Control Level 1, 2, and 3 should be tested in conjunction with patient plasmas. Level 1 is a lyophilized normal plasma. Levels 2 and 3 are adjusted to mimic moderately and severely deficient plasmas, respectively. Normal and abnormal controls should be run at the initiation of testing each day and at least once each shift, or with each group of assays. Controls should also be tested with each reagent change or major instrument adjustment. Each laboratory should establish a control group range to represent the allowable variation in day to day performance for each control.

VII. Results

Report clotting times for each plasma to the nearest 0.1 second. A Normal Reference Range can also be reported for comparison. Do not report patient values relative to commercial control plasma clotting times. Controls are intended only for quality assurance of the test system.

Determination of INR

An undesirable consequence of oral anticoagulant therapy may be a tendency to bleed unnecessarily. In order to maximize the desired therapeutic effects and minimize bleeding, the World Health Organization (WHO) has recommended a procedure to standardize testing and treatment. This procedure is based on the International Normalized Ratio (INR).^{6,7}

The INR is calculated using the ratio of the patient PT to the mean of a normal reference range (mean_{NRR}) according to the following mathematical relationship:

$$\text{INR} = \left(\frac{\text{Patient PT}}{\text{mean}_{\text{NRR}}} \right)^{\text{ISI}}$$

For example, with an ISI of 1.00 and a mean normal of 12.0 seconds, the INR for a PT of 40.0 seconds is calculated as follows:

$$\text{INR} = \left(\frac{40.0}{12.0} \right)^{1.00} = (3.33)^{1.00} = 3.33$$

The International Sensitivity Index (ISI) is a measure of a thromboplastin/instrument sensitivity to coagulation factors. ISI values are assigned by comparison to a primary reference material. High sensitivity reagents have low ISI values. According to WHO recommendations, INR values above 5.5 place the patient at unnecessary risk for bleeding complications. It is generally advised that patients on stabilized oral anticoagulant therapy should be maintained at an INR of 2.0 – 3.5, depending on the clinical indication.² The lot specific ISI value for Thromboplastin-DS can be found on the kit box label. For additional instrument ISI values, call Fisher Diagnostics Technical Services.

VIII. Limitations

The biochemistry of coagulation involves a series of reactions that are influenced by many pre-test conditions. These variables must be controlled to obtain reproducible results.³ It has been found that, on occasion, patient samples with INR values above the recommended therapeutic range of 2.0-3.5² may result in a “Coag Error 3” when using ACL instrumentation. If an error message is obtained, the sample should be tested using Thromboplastin-DS on an alternate method.

Technique:

- pH will increase if plasma is open to air. Store samples stoppered in plastic or siliconized glass.
- Plasma held at 4-8°C may undergo cold activation resulting in significant shortening of the PT.⁴
- Thromboplastin-DS was designed to work at 37°C ± 0.5°C. Frequently check the temperature of all heating elements.
- All labware must be clean and free of trace amounts of detergents.
- Always follow instrument manufacturer's instructions for proper maintenance.

Interfering Substances:

- Sodium oxalate, EDTA, and heparin are not suitable anticoagulants.
- The PT may be prolonged by substances such as oral contraceptives, corticosteroids, EDTA, asparaginase, clofibrate, erythromycin, ethanol, tetracycline, and anticoagulants such as heparin and warfarin.⁵
- The PT may be shortened by substances including antihistamines, butabarbital, caffeine, oral contraceptives, phenobarbital, and vitamin K.⁵

IX. Expected Values

In multi-center studies, when Thromboplastin-DS was evaluated on a normal population, the following results were obtained:⁹

Instrument	PT Mean(secs)	Range (+/-2SD)	N
MLA™ Electra 1000C™	13.2	11.4-15.0	40
MLA™ Electra 900C™	13.7	12.4-15.0	20
IL ACL™ 300/3000+	10.5	8.9-12.1	61
Amelung KC 10™	12.7	9.3-14.2	20
Pacific Hemostasis ThromboScreen 400C	13.5	12.2-14.8	38
Pacific Hemostasis ThromboScreen 200	13.5	12.0-15.1	60

These values are intended as a guideline only. Each laborato-

ry should establish a Normal Reference Range (NRR) using instrumentation, blood collection methods, and testing techniques used in that laboratory. The NRR should be reestablished or at least verified when changing lot numbers of the same reagent.^{3,6} A new NRR should be established with any change in reagents, instrumentation, blood collection techniques, or anticoagulant.

The clotting time of abnormal plasmas will depend on the ISI of the reagent lot in use.

X. Performance Characteristics

Precision: Precision of Prothrombin Time results is dependent on many factors, such as the instrument, technique and the reagent in use. Thromboplastin-DS precision was assessed by testing a normal and abnormal plasma on several different instruments. A summary of the results follows.¹⁰

Summary of Within-run Precision Studies, % CV (N = 20)

Sample	MLA Electra 1000C	ThromboScreen 400C	ThromboScreen 200	Amelung KC10
Normal	1.1%	1.9%	1.9%	2.9%
Abnormal	2.8%	2.5%	2.3%	1.1%

Sensitivity: Thromboplastin-DS detects deficiencies in the extrinsic pathway as determined by the Prothrombin Time test. Factor sensitivity testing was performed by diluting pool normal plasma with factor deficient plasmas such that the final factor concentration ranged from 10-100%. PT testing of these samples was performed on the MLA-1000C instrument.¹¹

% Factor	Prothrombin Time (secs)			
	Factor II	Factor V	Factor VII	Factor X
100	11.6	11.6	11.8	11.7
50	11.6	13.2	12.6	12.8
40	11.7	13.9	12.8	13.3
30	12.3	14.9	13.5	14.1
20	12.8	15.9	13.9	14.8
10	14.1	18.3	15.2	17.0

Correlation: Correlation studies were performed against two other sensitive thromboplastin reagents by performing PT testing on normal and abnormal samples. Testing was performed on the Stago STA instrument.¹²

	PT Correlation	INR Correlation
Thromboplastin DS vs. Reagent A, N = 49	R = 0.98 y = 1.16x + 1.30	R = 0.98 y = 0.89x + 0.05
Thromboplastin DS vs. Reagent B, N = 49	R = 0.95 y = 1.01x + 2.20	R = 0.95 y = 0.82x + 0.10

XI. References

1. Errichetti, A.M., Holden, A., Ansell, J.: *Management of Oral Anticoagulant Therapy: Experience with an Anticoagulation Clinic*. Arch Inter Med. 144:1966-68, 1984.
2. Hirsh, J., Dalen, J.E., Deykin, D., Poller, L.: *Oral Anticoagulants: Mechanisms of Action, Clinical Effectiveness, and Optimal Therapeutic Range*. Chest 102(Suppl): 312S-316S, 1992.
3. NCCLS: *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays; Approved Guideline*. NCCLS document H21-A4. NCCLS, Wayne, PA, 2003.
4. Palmer, R.N., Gralnick, H.R.: *Inhibition of the Cold Activation of Factor VII and the Prothrombin Time*. Am J Clin Path. 81: 618-622, 1984.
5. Young, D.S., Thomas, D.W., Friedman, R.B., et al: *Effect of Drugs on Clinical Laboratory Tests*. Clin Chem 18:1041, 1972.
6. Dalen, J.E., Hirsh, J.: *American College of Chest Physicians and the National Heart, Lung, and Blood Institute National Conference on Antithrombotic Therapy*. Arch Inter Med. 146:462-472, 1986.
7. Palaereti, G., Coccheri, S., Poggi, M., et al: *Oral Anticoagulant Therapy Control: Evidence that the INR Expression Improves the Interlaboratory Comparability of Results*. The Bologna Oral Anticoagulant Control Exercise. Thromb Haemostasis 58:905-910, 1987.
8. Stability data found in DHF.
9-12. Data found in 510(K) file.

Ordering Information

Cat. No.	Description	Contents
100354	Thromboplastin-DS	10 x 4 mL
100362	Thromboplastin-DS	10 x 10 mL
100353	Thromboplastin-DS	10 x 2 mL
100244	Reconstitution Fluid	10 x 10 mL

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