THROMBOPLASTIN PT

KIT FOR DETERMINATION OF PROTHROMBIN TIME (PT)

Kit name	Kit size	Cat. No
THROMBOPLASTIN PT- 4	10 x 4 ml	K-220
THROMBOPLASTIN PT-10	10 x 10 ml	K-250

INTRODUCTION

The Prothrombin Time test, as originally devised by Quick has been widely used for a number of years as a pre-surgical screen for assessing certain coagulation factors and in monitoring oral anticoagulant therapy.

This test will be prolonged in patients with acquired or congenital disorders that reduce the activity of factors I, II, V, VII, and X.

The PT is also widely used to monitor oral anticoagulant therapy. 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 Prothrombin Time test is also used in the quantitative determination (Factor Assays) of Factors II, V, VII and X.

METHOD PRINCIPLE

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.

REAGENTS

Package

	THROMBOPLASTIN PT-4	THROMBOPLASTIN PT-10
THROMBOPLASTIN PT	10 x 4 ml	10 x 10 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package.

Working reagent preparation and stability

Reconstitute THROMBOPLASTIN PT with distilled water according to the vial label (4 ml or 10 ml). Swirl gently and let the vial stand undisturbed for 15 minutes at room temperature. Do not invert the vial or mix vigorously.

After reconstitution, the reagent when stored stoppered is stable for 7 days at 2-8ºC, 8 hours at 37°C. Mix gently before each use. **Do not freeze.**

Concentrations in the test

rabbit brain tissue	< 0.09%
sodium azide	0.08%
buffers	2%

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- The reagents contain sodium azide. Avoid contact with skin and mucous membranes.
- THROMBOPLASTIN PT reagents are designed to work at 37°C. Frequently check the temperature of all heating elements.

ADDITIONAL EQUIPMENT

- a manual, mechanical or photo-optical means of clot detection;
- timer;
- control plasmas;
- general laboratory equipment.

SPECIMEN

A. Anticoagulant- sodium citrate - 3.2% (0.105M).

B. Specimen collection:

1. Obtain venous blood.
2. Immediately mix 9 parts blood with 1 part anticoagulant, mix well by inversion of tube.
3. Centrifuge the specimen at 1000 rcf for 15 min.
4. Remove plasma from the tube within 60 min using a plastic pipette and store in a plastic tube.
5. Test plasma sample within 2 hours, otherwise store frozen and thaw just prior to use.

Plasma pH will increase if exposed to air. Store samples stoppered. Do not delay mixing the blood with anticoagulant. Avoid foaming the specimen. Use only plastic containers.

PROCEDURE

Kit is suitable for use with manual, mechanical or automated instrument for clot detection. See instrument manufacturers instructions for full details.

Manual assay

1. Prewarm THROMBOPLASTIN PT to 37°C.
2. Add 0.1 ml test plasma to cuvette and prewarm to 37°C.
3. Forcibly add 0.2 ml warmed THROMBOPLASTIN PT to the test plasma and start timer.
4. Note time for clot formation.
5. Perform duplicate determinations.

Calculation

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, such as: temperature, reagents, pipettes, instrument etc.

International Committee for Standardization in Hematology and the International Committee on Thrombosis and Hemostasis have agreed on recommendations for the reporting of Prothrombin Time results based upon an International Sensitivity Index (ISI) of Thromboplastin reagents and an International Normalized Ratio (INR).

Thromboplastin reagents are assigned an ISI value by calibration against an International Reference Preparation, which by definition has an ISI = 1.0. The ISI value assigned to commercial Thromboplastin reagents therefore defines a comparative slope, or relative sensitivity, in comparison to the Reference Thromboplastin. The lower the ISI value, the more "sensitive" the reagent. By knowing the ISI of a particular Thromboplastin reagent, the International Normalized Ratio (INR) can be calculated:

a) $$R = \frac{\text{patient PT}}{\text{NRR}}$$

b) $$\mathbf{INR} = \mathbf{R}^{\mathbf{ISI}}$$

R- Cloting Time Ratio
NRR- Normal Reference Range
INR- International Normalized Ratio
ISI- International Normalized Ratio

The lot specific ISI value for THROMBOPLASTIN PT can be found on the kit box label.

REFERENCE VALUES

Typical Normal Results for PT are 11-14 secs.

These values should only be used as a guideline. Each laboratory should establish a Normal Reference Range (NRR) using instrumentation, blood collection methods, and testing techniques used in that laboratory.

A new NRR should be established with any change in instrumentation, blood collection, techniques, anticoagulant and when changing to new lots of reagents.

QUALITY CONTROL

CONTROL PLASMA-NORMAL LEVEL (Cat. No K-100) CONTROL PLASMA-ABNORMAL LEVEL 1 and 2 (Cat. No K-101 and K-102) should be tested in conjunction with patient plasmas. It is recommended that Controls be run at least each shift and a minimum of once per 20 patient samples.

A Control Range should be established by the laboratory to determine the allowable variation in day to day performance of each Control Plasma.

Each laboratory should establish a control range for each control.

LIMITATIONS

A. Plasma samples with hematocrits outside the range of 20-55% may be improperly anticoagulated and should be adjusted appropriately.

B. Turbid, icteric, lipemic, or hemolyzed specimens may generate erroneous results.

C. Freezing and thawing plasma can affect results.

D. Acute inflammatory reactions can shorten PT results because of elevated fibrinogen.

E. Sodium oxalate, EDTA, and heparin are not suitable anticoagulants.

F. The PT may be prolonged by substances such as oral contraceptives, corticosteroids, EDTA, asparaginase, erythromycin, ethanol, tetracycline, and anticoagulants such as heparin and warfarin.

G. The PT may be shortened by substances including antihistamines, butabarbital, caffeine, oral cotraceptives, phenobarbital,and vitamin K.

PERFORMANCE CHARACTERISTICS

1. Precision:

Precision was assessed by testing the 20 samples a normal and abnormal plasma on several different instruments.

Instrument	Normal plasma	Abnormal plasma
Organon Teknika MDA	CV=0.8%	CV=0.9%
MLA Electra 1000C	CV=0.6%	CV=1.8%
Amelung KC 4A	CV=2.0%	CV=2.1%

2. Sensitivity:

Sensitivity was assessed by testing the factor deficient plasmas ranged from 0-100% on the MLA-1000C instrument.

% Factor	Prothrombin time (s)			
	Factor II	Factor V	Factor VII	Factor X
100	10.9	11.3	11.2	11.4
50	10.5	12.6	12.2	13.0
40	10.5	13.1	12.8	13.3
30	10.9	13.8	13.5	14.5
20	11.4	15.2	14.3	15.9
10	13.4	17.6	16.0	19.6
0	34.0	55.9	25.9	108.8

3. Correlation

A correlation between CORMAY reagent (y) and commercially available reagent (x) using 101 samples gave following results:

PT correlation	INR correlation
y = 0.7406x + 2.945 R = 0.97 (correlation coefficient)	y = 0.9795x + 0.0029 R = 0.96 (correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Quick, A.J., the Hemorrhagic Diseases and the Physiology of Hemostasis. Charles C. Thomas: Springfield, IL. 1942.
2. Quick, A.J., Hemorrhagic Diseases. Lea and Febiger: Philadelphia. 1957.
3. Langdell, R., Wagner, R., Brinkhous, K.: Effect of Antihemophilic Factor on One Stage Clotting Tests. J. Lab. Clin. Med. 41: 637, 1953.
4. Errichetti, A.M., Holden, A., Ansell, J.: Management of Oral Anticoagulant Therapy: Experience with an Anticoagulation Clinic. Arch Inter Med. 144:1966-68, 1984.
5. 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.
6. NCCLS: Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Genera Performance of Coagulation Assays; Approved Guideline. NCCLS document H21 -A3. NCCLS, Wayne, PA, 1998.
7. 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.
8. Young, D.S., Thomas, D.W., Friedman, R.B., et al: Effect of Drugs on Clinical Laboratory Tests. Clin Chem 18:1041, 1972.
9. NCCLS: One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline. NCCLS document H47-A. NCCLS, Wayne, PA, 1996.
10. Dalen, J.E., Hirsh, J.: American College of Chest Physicians and the National Heart, Lung, and BloodInstitute National Conference on Antithrombotic Therapy. Arch Inter Med. 146:462-472, 1986.
11. Palaereti, G., Coccheri, S., Poggi, M., etal: 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.

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