THROMBOPLASTIN LIQUID-10

KIT FOR DETERMINATION OF PROTHROMBIN TIME (PT)

Kit name	Kit size	Cat. No
THROMBOPLASTIN LIQUID-10	10 x 10 ml	K-228
THROMBOPLASTIN LIQUID-10	4 x 10 ml	K-229

INTRODUCTION

THROMBOPLASTIN LIQUID reagent is intended for use in a one-stage prothrombin time (PT) test on citrated human plasma. The PT test is a quantitative assay used in the general patient population for routine screening to detect deficiencies in the extrinsic pathway of coagulation. The PT test is also used to monitor oral anticoagulant therapy

The one-stage prothrombin time test (PT) has become the basic coagulation screening test for the diagnosis of congenital and acquired deficiencies of Factors II, V, VII and X (1,2).

Oral anticoagulant drugs inhibit hepatic synthesis of the vitamin K dependent clotting Factors II, VII, IX and X. Therefore, the prothrombin time test is appropriate to monitor oral anticoagulant therapy since it is sensitive to three of the four factors involved (3,4). It can be modified to quantitate factor levels using appropriate factor deficient substrate plasmas.

METHOD PRINCIPLE

Tissue thromboplastin, in the presence of calcium ions and Factor VII, activates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal anti-coagulated plasma, the clotting mechanism is initiated and a clot will form within a specified time period. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged. The degree of prolongation is proportional to the severity of single factor deficiency, or in a cumulative deficiency of all the factors involved.

REAGENTS

Package

	THROMBOPLASTIN LIQUID-10	THROMBOPLASTIN LIQUID-10
THROMBOPLASTIN LIQUID	10 x 10 ml	4 x 10 ml

Reagent should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

Working reagent preparation and stability

Reagent comes ready for use.

After opening, the original activity is stable for 14 days at 2 to 8°C.

DO NOT FREEZE**!**

Ingredients

The reagent contains a liquid saline extract of rabbit brain, calcium ions, preservatives and stabilizers.

Warnings and notes

- Product for in vitro diagnostic use only.
- The PT test should be used in a clinical laboratory by qualified laboratory personnel.
- After initial whole blood collection, during testing all test tubes, syringes and pipettes should be plastic.
- The reagent is a fine suspension of rabbit brain particles. Large flaky particles in the suspension or prolonged prothrombin times on testing normal plasma or controls may indicate product deterioration.

ADDITIONAL EQUIPMENT

- coagulation instrument or 37°C water bath;
- timer;
- control plasmas;
- general laboratory equipment.

SPECIMEN

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

B. Specimen collection:

1. Obtain venous blood.
2. Nine parts freshly collected whole blood should be immediately added to one part anticoagulant.
3. Centrifuge the specimen at 2500 x g for 15 minutes.
4. Immediately separate the plasma from the red blood cells using a plastic pipette and place it in a plastic test tube at 2 to 8°C until assayed.
5. Perform the prothrombin time test within 2 hours.

Before and during testing, the plasma sample should be maintained in the plastic tubes at 2 to 8°C to ensure stability of the factors. If testing is delayed for more than 2 hours, the plasma may be stored at -20°C for two weeks or at -70°C for up to one month. Frozen samples should be thawed rapidly at 37°C before testing.

PROCEDURE

Prothrombin time test may be done by accepted manual methods or by using mechanical or photo-optical coagulation instruments.

See instrument manufacturers instructions for full details.

Throughout the procedure, all test tubes, syringes, and pipettes should be plastic.

Manual assay

- Reconstitute the control plasmas according to the package insert included with the control.
- Perform all tests in duplicate.
- Prewarm THROMBOPLASTIN LIQUID to 37°C for at least 10 minutes.
- Prewarm 100 µl of the test plasma or control plasma for 2-3 minutes at 37°C.
- Add 200 µl THROMBOPLASTIN LIQUID to the plasma, simultaneously starting a stopwatch and record the time required for clot formation in seconds.

Calculation

The results of the prothrombin time tests should be reported to the nearest tenth of a second. Results greater than the upper limits of the range should be considered abnormal and follow-up testing should be performed. PT values below the lower limits of the range may indicate a compromised sample, and a new sample should be collected.

The World Health Organization (WHO) recommends the use of the International Normalized Ratio (INR) based upon an International Sensitivity Index (ISI). This allows patient results to be compared between different laboratories that may be using reagents with different sensitivities.

The INR is calculated by using the ratio of the Patient PT to the mean of the normal reference range raised to the power of the reagent ISI.

$$INR = (\text{Patient PT} / \text{NRR})^{ISI}$$

NRR-Normal Reference Range

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.

The lot specific ISI value for THROMBOPLASTIN LIQUID-10 can be found on the kit box label.

REFERENCE VALUES
A reference range study was conducted using triplicate specimens from 120 normal health adults. Approximately equal numbers of males and females were used. The PT results were as follows:

Method	Mean [second]	Range for +/- 2 SD [second]
photo-optical	12.3	9.7 - 14.9
mechanical	12.6	11.1 - 14.1

These values should only serve as guidelines. Because differences may exist between instruments, laboratories, and local populations, it is recommended that each laboratory establish its own reference range of expected prothrombin time results for each new lot.

QUALITY CONTROL
CONTROL PLASMA NORMAL (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. The normal and abnormal controls should be tested daily prior to performing tests on patient plasmas. Monthly quality control charts (Levy-Jennings) are recommended to determine the mean and standard deviation of each of the daily control plasmas.
If the controls do not perform within their reference ranges, patient results should be considered invalid and not reported.
Each laboratory should establish a quality control program that includes normal and abnormal controls to evaluate instrument, reagent and technologist performance.

LIMITATIONS
A. Expected values for the prothrombin time test will vary from one laboratory to another, depending on several variables include the method of clot detection, temperature, pH, sample collection technique, type of anticoagulant and time and method of plasma storage.
B. Laboratories should establish their own expected values for patients and well defined performance standards for control plasmas.
C. The use of icteric, lipemic, or hemolyzed sample should be avoided due to possible interference especially when using photo-optical instruments.
D. The impact of other therapeutic drugs, in addition to oral anticoagulant therapy, can influence interpretation of PT test results.
E. Obtaining an accurate patient history and noting specific drug therapies can help in the proper understanding of the potential impact on laboratory test results.
F. The presence of heparin as a contaminant in the patient sample must always be considered when an abnormal result is obtained.

PERFORMANCE CHARACTERISTICS
The studies were done using photo-optical coagulation instrument K3002 OPTIC.

- **Precision**

Precision studies were performed using THROMBOPLASTIN LIQUID of the same lot and normal and abnormal control plasmas. Results for INR are shown below:

Repeatability (run to run) n = 20	Mean	SD	CV
normal level	0.90	0.03	3.16
abnormal level 1	1.29	0.03	1.95
abnormal level 2	3.64	0.12	3.37

Reproducibility (day to day) n = 28	Mean	SD	CV
normal level	1.14	0.06	5.19
abnormal level	4.30	0.30	6.91

- **Correlation**

A comparison between THROMBOPLASTIN LIQUID (y) and commercially available assay (x) using 42 samples. The linear regression equation and coefficient of correlation R of the INR values are reported:
$y = 0.865 x + 0.0684$;
$R = 0.9876$ (R - correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

1. Quick A.J., The Prothrombin Time in Hemophilia and in Obstructive Jaundice. J. Biol. Chem.: 109,73-74; 1935.
2. Biggs R. ed , Human Blood Coagulation Hemostasis and Thrombosis Second Ed. Blackwell Scientific Publications, London 1976.
3. Peterson C.E., Kwaan H.C., Current Concepts of Warfarin Therapy, Arch Intern. Med. 146: 581-584, 1986.
4. Loeliger E.A.: ICEH/ICTH Recommendations for Reporting Prothrombin Time in Oral Anticoagulant Control, Throm. Haemost. 53: 155-156,1985

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