

# THROMBOPLASTIN REAGENT FOR PROTHROMBIN TIME TEST

Cat. No. 5380, 5381, & 5382

Helena  Laboratories

Helena Thromboplastin Reagent is intended for the performance of the one stage prothrombin time test and assays for Factors II, V, VII and X.

## SUMMARY

The first standardized one-stage prothrombin time test was developed by Dr. Armand Quick in 1935.<sup>2</sup> It has become the basic coagulation screening test for the diagnosis of congenital and acquired deficiencies of the extrinsic pathway involving Factors I, II, V, VII and X.<sup>1,2</sup>

Oral anticoagulants such as Coumarin and Dicumarol interfere with the liver's production of the vitamin K dependent clotting factors II, VII, IX and X. Therefore, the prothrombin time test is used to monitor oral anticoagulant therapy since it measures three of the four factors involved.

## PRINCIPLES OF THE PROCEDURE

Tissue thromboplastin, in the presence of calcium ions, is an activator which initiates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal anticoagulated plasma, the clotting mechanism is initiated leading to formation of a fibrin clot. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged depending on the severity of the deficiency.

## REAGENTS

### Thromboplastin Reagent

**Ingredients:** The reagent contains dehydrated rabbit brain extract in saline with calcium ions, preservatives and stabilizers.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.**

**Preparation for Use:** Reconstitute each vial of reagent with deionized water according to the instructions on the vial label. Mix vigorously to ensure complete dissolution.

**Storage and Stability:** Unreconstituted reagent should be stored at 2 to 6°C and is stable until the expiration date indicated on the vial. After reconstitution, the original activity is stable for seven (7) days at 2 to 6°C, 24 hours at room temperature, or 8 hours at 37°C.

**Signs of Deterioration:** The reagent is a fine suspension of rabbit brain particles. Large flaky particles in the suspension and prolonged prothrombin times on normal plasma or controls

may be indicative of product deterioration. There is no standard of potency for a thromboplastin reagent.

## INSTRUMENTS

Any high quality electro-mechanical or photo-optical coagulation instrument may be used such as the Cascade® 480 (Cat. No. 1430), the Cascade M (Cat. No. 1710) or the Cascade M-4 (Cat. No. 1711).

## SPECIMEN COLLECTION AND HANDLING:

Throughout the procedure for determination of prothrombin times, all test tubes, syringes and pipettes must be plastic or siliconized glass. Blood collected into inadequately siliconized tubes may demonstrate shortening of prothrombin time values, thus leading to increased dosage of anticoagulants. This leads to incidences of clinical bleeding.

**Specimen:** Plasma obtained from whole blood collected with 3.2% or 3.8% sodium citrate as an anticoagulant is the specimen of choice.

**Specimen Collection:** Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. According to NCCLS guidelines, blood specimens with hematocrits (HCT) of < 55% should be obtained by adding 9 parts of freshly collected whole blood to one part anticoagulant.<sup>7</sup> For blood specimens with hematocrits > 55%, adjust the amount of whole blood added to the anticoagulant according to the following formula.<sup>3</sup>

$$\frac{\text{Parts whole blood to}}{\text{one part anticoagulant}} = \frac{0.6}{(1-\text{HCT})} \times 9$$

Particular care should be taken when using evacuated test tubes. These tubes are designed to draw 9 parts blood to 1 part anticoagulant. If the hematocrit is determined to be abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant.

**Specimen Preparation:** Centrifuge the whole blood specimen at 1600 - 2000 x g for 10 minutes. A refrigerated centrifuge is preferred. Immediately separate the plasma from the red blood cells and place it in a plastic test tube with a cap. Perform the prothrombin time assay within 2 hours.

**Storage and Stability:** Prior to testing, the plasma sample should be maintained in the plastic tubes at 2 to 6°C. If testing is delayed for more than 2 hours, the plasma may be stored at -20°C or colder for up to one month. Thaw at 37°C quickly prior to testing.

## PROCEDURE

**Materials Provided:** Materials needed for prothrombin time tests are provided:

	Cat. No.
Thromboplastin Reagent (10 x 4.0 mL)	5380
Thromboplastin Reagent (10 x 10 mL)	5381
Thromboplastin Reagent (10 x 20 mL)	5382

### Materials Needed but not Provided:

Coagulation Instrument: Helena Cascade 480 (Cat. No. 1430), Cascade M (Cat. No. 1710) or the Cascade M-4 (Cat. No. 1711) is recommended.

Reaction cups

Pipetting device to deliver 0.2 mL or 0.1 mL

Control plasmas: Helena Norm-Trol (Cat. No. 5186), Ab-Trol I (Cat. No. 5187), Ab-Trol II (Cat. No. 5183) and Coagulation S.A.R.P. (Cat. No. 5185) are recommended

Plastic test tubes (12 x 75 mm)

37°C Heat Block or Water Bath

Centrifuge

3.2% or 3.8% Sodium Citrate for blood collection

## STEP-BY-STEP METHOD

Throughout the procedure, all test tubes, syringes, and pipettes, must be plastic or siliconized glass.

1. Collect and prepare the blood specimen according to directions outlined in SPECIMEN COLLECTION AND HANDLING.
2. Reconstitute the control plasmas according to the package insert included with the control.
3. Prepare the reagents for use in the procedure according to the reconstitution instructions in the REAGENT section.
4. Perform all tests in duplicate. Calculate the mean clotting time of the duplicate determinations to the nearest 0.1 second. Use the enclosed conversion table to report out the INR value for each patient tested, or the Cascade 480 will automatically calculate the INR value.

### I. Manual and Electromechanical Method

1. Prewarm the reagent to 37°C for at least 10 minutes.
2. Prewarm 0.1 mL of the test plasma or control for 2-3 minutes at 37°C.
3. Add 0.2 mL working Thromboplastin Reagent to the plasma, and note the time required for clot formation.

## II. Automated Methods

If using the Cascade 480, Cascade M or the Cascade M-4 to perform this test, refer to the appropriate Operator's Manual for detailed instructions.

**Quality Control:** Each laboratory should establish a quality control program that includes normal and abnormal controls to evaluate instrument, reagent and technologist performance. The quality controls should be performed prior to performing each batch of tests on patient plasmas and with each change of personnel. Monthly quality control charts provided by the College of American Pathologists Q.A.S. program, or equivalent, are recommended to determine the mean and standard deviation of each control. The controls Norm-Trol (Cat. No. 5186), Ab-Trol I (Cat. No. 5187), Ab-Trol II (Cat. No. 5183) and Coagulation S.A.R.P. (Cat. No. 5185) are available from Helena Laboratories. If the controls do not perform as expected, patient results should be considered invalid.

## REFERENCE VALUES

A reference range should be established by each laboratory using a representative population.

Prothrombin times were done in duplicate on the Cascade 480, on a group of 39 healthy individuals.

$$\bar{X} \pm 2 \text{ SD} = 10.4 - 12.4 \text{ seconds}$$

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 range of expected prothrombin time.<sup>6</sup>

## LIMITATIONS

Reference values for the prothrombin time test will vary from one laboratory to another, depending on individual techniques. The method of clot detection, temperature, pH, collection technique, type of anticoagulant and time and method of plasma storage influence results. Therefore, laboratories should establish their own expected values for patients and well defined performance standards for the control. Refer to the SPECIMEN COLLECTION AND HANDLING Section for interfering substances.

The increasing use of drugs in medical therapy, in addition to oral anticoagulation therapy, makes interpretation of laboratory test results more difficult. Obtaining an accurate patient history and noting specific types of drug treatment aid in the proper understanding of its effect on laboratory test results.<sup>4</sup>

The use of heparin or EDTA as an in-vitro anti-coagulant may give invalid prothrombin time results.<sup>5</sup>

## PERFORMANCE CHARACTERISTICS

### I. Precision

Within Run: Reproducibility studies were done using a normal and an abnormal control plasma and a single lot of reagent with the following results.

	Normal	Abnormal
N	36	36
$\bar{X}$	12.1	20.3
2 SD	0.25	0.5
CV%	1.1	1.2

Between Run: The above stated study was also tested with an additional lot of reagent.

	LOT A		LOT B	
	Normal	Abnormal	Normal	Abnormal
N	36	36	36	32
$\bar{X}$	12.1	20.3	12.4	20.4
2 SD	0.25	0.5	0.22	0.4
CV%	1.1	1.2	0.9	1.0

### II. Comparison

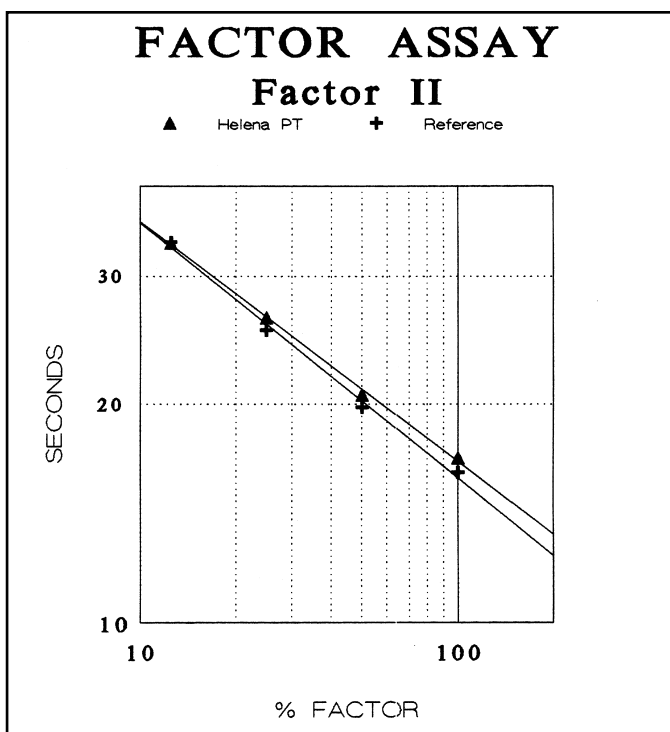
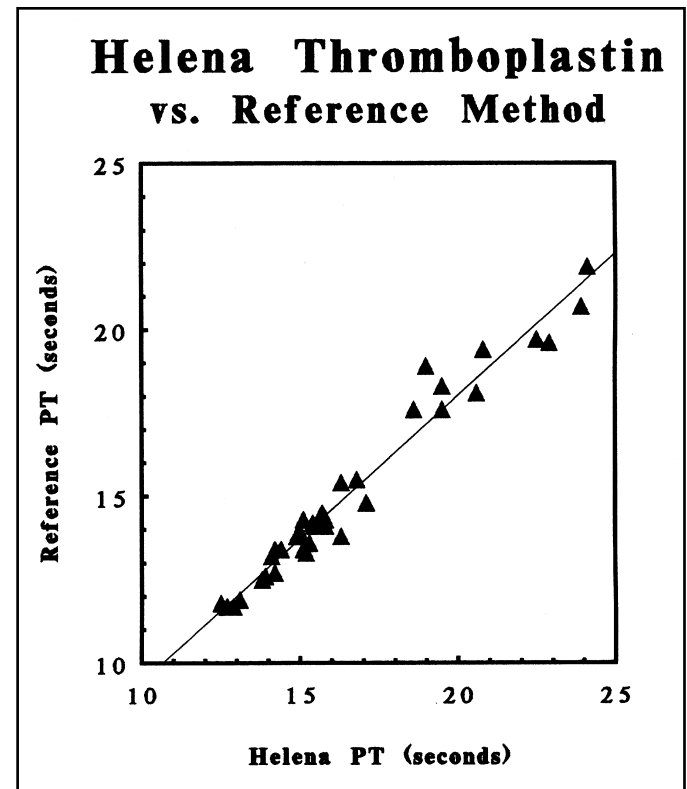
Several comparison studies have been performed using the Helena Thromboplastin Reagent and the reference method reagent.

#### A. Factor Assays

Comparison studies on Factor Assays were performed on Factors II, V, VII and X. A typical comparison is shown here for Factor II.

### B. Oral Anticoagulant Monitoring

Another comparison study was done with 33 oral anticoagulated patients on 5 control plasmas comparing PT's using Helena's and a reference method. Each sample was done in duplicate. The means were used to determine a linear regression equation of  $Y = 0.84 + 0.86X$  with a correlation coefficient of 0.981, where X is the Helena reagent and Y is the reference reagent.



## BIBLIOGRAPHY

1. Biggs, Rosemary, Ed., Human Blood Coagulation, Haemostasis and Thrombosis, 2nd Ed., Blackwell Scientific Publications, London, 1976.
2. Quick, A.J., Hemorrhagic Diseases and Thrombosis, 2nd Ed.. Lea and Febiger, Philadelphia, 1966.
3. Triplett, D.A., Ed., Standardization of Coagulation Assays: An Overview, Coll of Am Path, Skokie, Ill, p 4, 1982.
4. Lubran, M., The Effects of Drugs on Laboratory Values, *Med Clin of N Am* 53(1): 211-212,1969.
5. Young, D.S., et al., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington, D.C., 1990.
6. NCCLS, How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory Proposed Guideline - C28P, 1992
7. NCCLS, Collection, Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, 2nd ed. H21-A2, 1991.

## Thromboplastin Reagents

Helena Thromboplastin Reagent	Cat. No.
Thromboplastin Reagent (10 x 4 mL)	5380
Thromboplastin Reagent (10 x 10 mL)	5381
Thromboplastin Reagent (10 x 20 mL)	5382

## EQUIPMENT AND SUPPLIES

Coagulation S.A.R.P. (10 x 1 mL)	5185
Norm-Trol Coagulation Control (10 x 1.0 mL)	5186
Ab-Trol 2 Coagulation Control (10 x 1.0 mL)	5187
Ab-Trol 3 Coagulation Control (10 x 1.0 mL)	5183
Cascade® 480	1430
Cascade® M	1710
Cascade® M-4	1711

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