The Helena Thromboplastin-LI Manchester Reagent is intended for the performance of the one-stage prothrombin time test and assays for Factors II, V, VII and X. It is recommended for laboratories desiring a more sensitive ISI (1.0-1.2) for monitoring oral anticoagulant therapy.

SUMMARY
The first standardized one-stage prothrombin time test was developed by Dr. Armand Quick in 1935. 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.

Oral anticoagulants such as Coumadin 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 and a solid 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 depending on the severity of the deficiency.

REAGENT
1. Thromboplastin-LI Manchester Reagent

Ingredients: The reagent contains liquid rabbit brain thromboplastin, preservatives and stabilizers.

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

Preparation for Use: Mix one vial of reagent with one vial of Calcium Chloride. Smaller equal aliquots may be used, if desired. Mix to ensure complete suspension.

Storage and Stability: Reagent should be stored at 2 to 6°C and is stable until the expiration date indicated on the vial. The mixed working reagent activity is stable for seven (7) days at 2 to 6°C or 24 hours at room temperature.

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.

2. Calcium Chloride

Ingredients: The reagent contains 0.025 M calcium chloride.

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

Preparation for Use: Mix equal volumes of CaCl₂ with reagent. Mix to ensure complete suspension.

Storage and Stability: The CaCl₂ should be stored at 2 to 6°C and is stable until the expiration date indicated on the vial.

Signs of Deterioration: Turbidity, precipitation or prolonged prothrombin times on normal plasma or controls may be indicative of product deterioration. There is no standard of potency for a thromboplastin reagent.

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

SPECIMEN COLLECTION AND HANDLING
Throughout the procedure for determination of prothrombin times, all test tubes, syringes and pipettes must be plastic or siliconized glass.

Specimen: Plasma obtained from whole blood collected with 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. For blood specimens with hematocrits greater than 55%, adjust the amount of whole blood added to the anticoagulant according to the following formula. 

\[
\text{parts whole blood to one} = 0.6 \times 9 - \text{part anticoagulant} \times \text{(1 - HCT)}
\]

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.

**STORAGE**

Preparation: Centrifuge the whole blood specimen at 1600 - 2000 x g for 10 minutes. Immediately separate the plasma from the red blood cells, and place it in a plastic test tube with a cap at 2 to 6°C until assayed. Perform the prothrombin time assay within 2 hours.

**QUALITY CONTROL**

Each laboratory should establish its own range of expected prothrombin time. It is recommended that each laboratory establishes its own range of expected prothrombin time. 

**RESULTS**

The results of the prothrombin test should be reported to the nearest 1/10 of a second. The normal range (usually \( X \pm 2 \) Standard Deviations) for each individual laboratory should be established. Results greater than the upper limits of the normal range should be considered abnormal, and follow-up testing should be performed. PT values less than the lower limits of the normal range should be reported on a new blood sample.

**LIMITATIONS**

Expected 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.

The presence of heparin or EDTA as an in-vitro anticoagulant may give invalid prothrombin time results.

**PERFORMANCE CHARACTERISTICS**

**I. Precision**

Within-Run and Between-Run precision studies were done on a normal control and abnormal control.

<table>
<thead>
<tr>
<th>RUN</th>
<th>WITHIN-RUN</th>
<th>BETWEEN-RUN</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RUN #1</td>
<td>RUN #2</td>
</tr>
<tr>
<td>Norm-Trol 2</td>
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<td>20</td>
</tr>
<tr>
<td>mean 28.8</td>
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<td>SD 0.75</td>
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<td>%CV 2.62</td>
<td>1.41</td>
<td>1.23</td>
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**BIBLIOGRAPHY**

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. For blood specimens with hematocrits greater than 55%, adjust the amount of whole blood added to the anticoagulant according to the following formula.

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

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. Immediately separate the plasma from the red blood cells, and place it in a plastic test tube with a cap at 2 to 6°C until assayed. 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 quickly at 37°C prior to testing.

**PROCEDURE**

**Materials provided:** Materials needed for prothrombin time tests are provided:
- Cat. No. 5269
  - 10 x 10 mL Thromboplastin-LI Manchester Reagent
- 10 x 10 mL Calcium Chloride

**Materials needed but not provided:**
- Reaction cups
- Pipetting device to deliver 0.2 mL and 0.1 mL
- Control plasmas: Helena Norm-Trol (Cat. No. 5186), Ab-Trol 2 (Cat. No. 5187) and Ab-Trol 3 (Cat. No. 5183) 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 reagents for use in the procedure according to the 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 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 or Cascade M 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 normal and abnormal controls should be tested daily prior to performing tests on patient plasmas. Monthly quality control charts provided by Helena’s Quality Assurance Review (QAR) program are recommended to determine the mean and standard deviation of each control. The Helena controls Norm-Trol 1 (Cat. No. 5186), Ab-Trol 2 (Cat. No. 5187), and Ab-Trol 3 (Cat. No. 5183) are recommended. If the controls do not perform as expected, patient results should be considered invalid.

**REFERENCE VALUES**

A reference range study was conducted by Helena Laboratories using duplicate specimens from 25 healthy individuals. The results were as follows:

\[ n = 25 \]
\[ X \pm 2 \text{SD} = 12.7 \pm 1.1 \text{seconds} \]

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.

**RESULTS**

The results of the Prothrombin test should be reported to the nearest 1/10 of a second. The normal range (usually \( X \pm 2 \text{ Standard Deviations} \)) for each individual laboratory should be established. Results greater than the upper limits of the normal range should be considered abnormal, and follow-up testing should be performed. PT values less than the lower limits of the normal range should be repeated on a new blood sample.

**LIMITATIONS**

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**Thromboplastin-LI Manchester Reagent**

Cat. No. 5269
10 x 10 mL Thromboplastin-LI Manchester Reagent
10 x 10 mL Calcium Chloride

**EQUIPMENT AND SUPPLIES**

- 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

For Sales, Technical and Order Information, and Service Assistance, call 800-231-5663 toll free.

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