

Thrombin Clotting Time Reagent

Cat. No. 5377

Helena  Laboratories

For use in determination of thrombin clotting times on plasma using semi-automated and automated methods.

SUMMARY

The thrombin clotting time (TCT), also known as the thrombin time, is a rapid screening test for abnormalities in plasma fibrinogen levels. The test is commonly applied to detect various sources of interference with normal blood coagulation, including fibrin(ogen) degradation products (FDPs) and heparin.^{1,2}

PRINCIPLE

Plasma fibrinogen is cleaved by thrombin (activated factor II, provided by the TCT reagent) to form fibrin, which polymerizes to form a fibrin clot. The time required for these reactions under standardized conditions (0.2 mL plasma + 0.1 mL of TCT reagent, 37°C) is reported as the thrombin clotting time. Clot detection can be by mechanical, or photo-optical (e.g., Cascade 480) measurement. Prolongation of the thrombin clotting time can be taken as a qualitative indication of abnormal fibrinogen levels (either high or low), or of the presence of interfering substances such as FDPs or heparin. Quantitative evaluation of these possible causes of TCT prolongation should be pursued with appropriate follow-up testing, e.g., APTT or chromogenic assay for heparin, Clauss fibrinogen assay for quantitative fibrinogen determination, or specific assays for FDPs. Alternative follow-up procedures may include heparin neutralization with protamine sulfate or polybrene¹, or normal plasma mixing studies³ or reptilase assay¹ to distinguish between hypofibrinogenemia and FDP effects.

REAGENT

1. Thrombin Clotting Time Reagent (5377): The reagent is a lyophilized preparation of approximately 15 NIH units/vial of bovine thrombin. The reconstituted reagent contains approximately 3 NIH units/mL of bovine thrombin. Buffers and stabilizers have been added.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY

Preparation for Use: Reconstitute the reagent with 5.0 mL of distilled or deionized water. Gently invert the vial several times to thoroughly dissolve the lyophilized reagent plug.

Storage and Stability: Helena Thrombin Clotting Time Reagent should be stored at 2 to 6°C and is stable until the date indicated on the vial.

Avoid contamination of reagent by following appropriate laboratory cleanliness procedures. After reconstitution, the reagent is stable for 8 hours when stored at 2 to 6°C.

Signs of Deterioration: The lyophilized reagent should be a white to off-white plug, or pieces of a plug. After reconstitution, TCT should form a clear, colorless solution. Appearance other than described, or failure of normal plasma or controls to fall within established laboratory quality control ranges, may be indicative of product deterioration.

INSTRUMENT

Any high quality electro-mechanical or photo-optical coagulation instrument designed for performing TCT measurements may be used. The Helena Cascade[®] 480 is recommended.



SPECIMEN COLLECTION AND HANDLING

Throughout the procedure for determination of thrombin clotting times (TCT), all test tubes, syringes and pipettes must be plastic or siliconized glass.

Specimen: Plasma obtained from whole blood collected with sodium citrate as an anticoagulant. The concentration of the sodium citrate should be 3.8% (0.129 M) or 3.2% (0.109 M).

Specimen Collection: Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Avoid contamination with tissue fluids, hemolysis or air bubbles.

Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. For blood specimens with hematocrits (HCT) of 40-50% (normal),

9 parts of freshly collected whole blood should be immediately added to one part anticoagulant. For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula:⁴

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

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 abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant. For greater stability of the specimen, an acid citrate anticoagulant solution should be prepared and utilized.

The anticoagulant is prepared as follows:⁵

3 parts 0.1 M sodium citrate
2 parts 0.1 M citric acid

Specimen Preparation: Centrifuge the whole blood specimen at 1600-2000 x g for 10 minutes. Longer centrifugation time and/or greater g force has been recommended if samples are to be frozen.⁶ Immediately separate the plasma from the red blood cells and place it in a plastic test tube with cap. Perform the thrombin clotting time assay within 2 hours.

Storage and Stability: Prior to testing, the plasma sample should be stored in capped plastic test tubes at 2 to 6°C. If testing is delayed for more than 2 hours, plasma may be stored at -20°C or colder for up to one month. Thaw quickly at 37°C prior to testing but do not leave at 37°C for more than 5 minutes.

PROCEDURE

Materials Provided:

Cat. No. 5377: Reagent for 500 tests.
10 x 5 mL Thrombin Clotting Time (TCT) Reagent

Materials and Equipment Required but not Provided:

Coagulation Instrument: Helena Cascade 480 (Cat. No. 1430) is recommended
Pipettes to deliver 0.2 mL and also 0.1 mL for non-automated methods
Control Plasmas: Helena Norm-Trol 1 (Cat. No. 5186) is recommended
Centrifuge
Materials for specimen collection (see above)

STEP BY STEP METHOD

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

I. Electro-mechanical

1. Collect blood specimen according to directions in SPECIMEN COLLECTION AND HANDLING SECTION.
2. Centrifuge the anticoagulated whole blood specimen at 1600-2000 x g for 10 min.
3. While the blood specimen is in the centrifuge, reconstitute the control plasma according to the package insert included with the control.
4. Immediately after centrifugation, separate the plasma from the red blood cells and place in a plastic tube with cap at 2 to 6°C until assayed. The maximum storage time at 2 to 6°C is 2 hours.
5. Reconstitute Thrombin Clotting Time Reagent with 5.0 mL of deionized or distilled water.
6. Pipette 0.2 mL (Note: 0.2 mL of sample is required rather than the lesser volume used in tests such as PT or APTT) of the patient plasma or control plasma into the reaction cup. Equilibrate at 37°C for three (3) minutes.
7. Pipette 0.1 mL of TCT Reagent, equilibrated to room temperature, into the reaction cup containing patient plasma or control plasma while simultaneously starting the timer.
8. Perform all tests in duplicate. If the difference in results of duplicate tests is greater than the allowable variance established in your laboratory (typically 5%), repeat the assay.

II. Automated Methods

If using the Cascade 480 or other instrument to perform this test, refer to the appropriate Operator's Manual for instructions. (Note: 0.2 mL of sample is required rather than the lesser volume used in tests such as PT or APTT)

Quality Control: Each laboratory should establish a quality control program to evaluate instrument, reagent and technologist performance. The quality control should be performed with each run when performing tests on patient plasmas and with each change of personnel. Monthly quality control charts provided by Helena's Quality Assurance Review (QAR) program are recommended to determine the mean and standard deviation of the control. The Helena control Norm-Trol 1 (Cat. No. 5186) is recommended. If the control does not perform as expected, the patient values should be considered invalid.

RESULTS

The results of the TCT 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 (see PRINCIPLE above).

EXPECTED VALUES

A normal range study was conducted using frozen plasma samples from 66 normal adult donors (ap-

proximately equal numbers of males and females). Results for Helena TCT Reagent run on the Cascade 480 with samples from blood collected into evacuated collection tubes containing 3.8% sodium citrate were as follows:

Range \pm 2 S.D. = 11.6 - 20.7 seconds
 \bar{X} = 16.18 seconds
 S.D. = 2.27

Typically the normal range for electromechanical instruments (i.e. DataClot 2) runs approximately 1.5 seconds longer than the Cascade 480 range. These values should serve only as guidelines. Because differences may exist among instruments, laboratories and local populations, it is recommended that each laboratory establish its own range of expected values.

LIMITATIONS

Expected values for the TCT test will vary from one laboratory to another, depending on the technique used. The method of clot detection, temperature, sample pH, collection technique, concentration of anticoagulant and time and method of specimen storage are all very important. Thus, laboratories should establish their own expected values for patients and well defined performance standards for the control. In addition to the causes of TCT prolongation indicated in the SUMMARY and PRINCIPLE sections, a recent report has suggested that many systemic amyloidosis patients with bleeding complications may have a circulating inhibitor that prolongs the TCT.⁷ TCT's were normal in this study when performed on centrifuged and resuspended fibrinogen from the patient samples.⁷ Also, therapeutic levels of heparin may entirely abolish clotting in the TCT test, although neutralization with protamine sulfate or polybrene should correct the TCT'.

PERFORMANCE CHARACTERISTICS

I. PRECISION STUDIES

Within Run:

Precision studies were performed using five levels of heparinized plasma run on the Cascade 480 instrument to evaluate within run variation.

Results are shown below:

(TCT in seconds)

Mean	S.D.	C.V.	N
13.21	0.41	3.13%	40
18.21	0.87	4.79%	16
34.25	1.07	3.13%	20
38.25	1.33	3.49%	20
45.81	2.01	4.38%	20

Between Run:

Studies were performed using a control material run on the Cascade 480 instrument. The following results represent 5 runs of 8 de-

terminations each using 5 different vials of reagent. The testing was done on one day.
 (TCT in seconds)

Mean	S.D.	C.V.	N
13.21	0.32	2.39%	40

II. COMPARISON STUDIES

A. Comparison of Helena TCT Reagent and a commercially available reagent was performed using blood samples from individuals with normal clotting activity. The following results were obtained:

Instrument	Reagent	N	mean TCT (seconds) \pm 2 S.D.
Cascade 480	Helena TCT	33	15.8 + 4.9
Cascade 480	Comparison	33	13.4 + 4.6

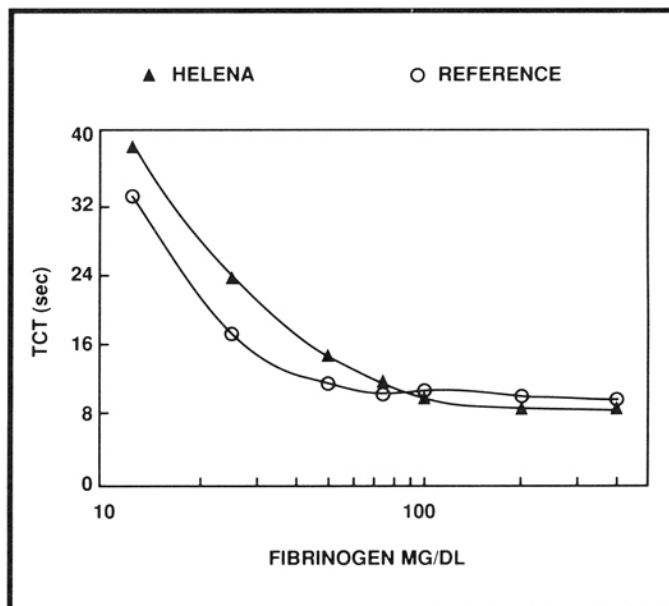
B. PATIENT TESTING COMPARISON

Comparison of Helena TCT Reagent and a commercially available reagent was performed using 33 blood samples with normal clotting activity and 31 samples with abnormal clotting activity due to either heparin therapy or fibrinogen abnormalities. A total of 64 samples, measured in duplicate, were examined. The following results were obtained:

slope = 1.204 Y = Helena
 intercept = 0.71 X = Reference
 correlation coeff. (R) = 0.952 Method

C. EFFECT OF FIBRINOGEN LEVEL

Comparison with a commercially available reagent was made on samples prepared with a wide variation of fibrinogen levels to illustrate the effect of fibrinogen on the Thrombin Clotting Time. Again, these values should serve only as guidelines.



BIBLIOGRAPHY

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7. Gastineau, D.A. et al., Inhibitor of the Thrombin Time in Systemic Amyloidosis: A Common Coagulation Abnormality, Blood 77: 2637-2640, 1991.

TCT REAGENT

	Cat. No.
Helena Thrombin Clotting Time Reagent	5377
Reagent for 500 tests 10 x 5 mL TCT Reagent	
EQUIPMENT AND SUPPLIES	
Helena Cascade 480	1430
COAGULATION A.R.P. (10 x 1.0 mL)	5185
Norm-Trol 1 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
Hep-Trol Coagulation Control (10 x 1.0 mL)	5189

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