REAGENTS

The HEMOCHRON TT rose-top test tube (A301) contains:

- lyophilized preparation of human thrombin, calcium salts, stabilizers and buffers
- Thimerosal (0.02%) is added as a preservative

The HEMOCHRON HNTT brown-top test tube (A401) contains:

- lyophilized preparation of human thrombin, calcium salts, protamine sulfate stabilizers and buffers
- Thimerosal (0.02%) is added as a preservative

A single vial of deionized, distilled water (1.5 cc) is provided with each test tube.

CAUTION: *These products include materials that have been prepared from human plasma or serum which have been tested using FDA licensed methods and found to be non-reactive for Human Immunodeficiency Virus (HIV) and for hepatitis B surface antigen (HBsAg). However, as no test method can offer complete assurance that infectious agents are absent, all specimens of human origin should be considered potentially infectious and handled with care.*

STORAGE AND STABILITY

When refrigerated (2-8° C), the TT and HNTT test tubes are stable until the marked expiration date. This product may also be stored at a controlled room temperature (15-30° C). Room temperature dating is to a maximum of two weeks, but must never exceed the marked expiration date. If stored at room temperature, redating on the enclosed label is necessary. The label should be affixed to the outside of the product box, covering the stamped expiration date. The test tubes should not be exposed to temperatures in excess of 37° C.

SPECIMEN COLLECTION AND HANDLING

Materials Provided

- HEMOCHRON TT rose-top or HEMOCHRON HNTT brown-top test tubes
- Deionized, distilled water

Materials Required

- HEMOCHRON *Response*, 8000, 801 or 401
- 3 cc syringes
- One syringe (adequate to deliver 1.0 cc of water)

Before performing any test, the user should refer to the appropriate HEMOCHRON operator's manual for detailed operating instructions.

The TT and HNTT tests can be used with either fresh or citrated whole blood. For fresh blood collection, adhere to the appropriate technique (A, B or C):

A. Indwelling venous blood-line (Do not obtain blood from a heparinized access line, or indwelling heparin lock):

1. Discontinue fluids drip, if required.
2. Use a two syringe technique - discard the first 5 cc draw. Obtain a 2 cc sample with the second syringe for testing.

B. Extracorporeal blood line port

1. Flush the extracorporeal blood access line by withdrawing and discarding 5 cc of blood.
2. Draw a 2 cc sample with a second syringe for testing.

C. Venipuncture

Obtain a 2 cc sample with a syringe.

NOTE: *Fresh whole blood samples must be tested immediately after collection. If the blood has been collected into a sodium citrate tube, it is important that the specimen be tested within one hour of drawing. Keep the specimen at room temperature - do not heat or ice.*

TEST PROCEDURE

NOTE: *The TT and HNTT test tubes and water vials must be at room temperature prior to rehydration. Once removed from the refrigerator, this may take up to 60 minutes. Testing in duplicate may improve the accuracy of results.*

1. Prior to collection of the blood sample, rehydrate each test tube to be used by adding 1 cc of the supplied deionized, distilled water. Use a syringe for accuracy to transfer the water into test tube directly through the stopper.
2. Gently agitate the test tube until the lyophilized preparation is dissolved.

NOTE: *For optimal performance, TT or HNTT tubes should be incubated at 37°C within 30 minutes after rehydration.*

3. Place the test tube in the test well and incubate for 180 seconds.

NOTE: *For optimal performance, TT or HNTT tubes should be used within 30 minutes after incubation. DO NOT incubate a second time.*

4. After 180 seconds have elapsed on the instrument, remove the TT or HNTT test tube from the wall.

NOTE: *It is important to remove the TT or HNTT test tube from the instrument to avoid over-incubation.*

5a. *For fresh whole blood*: Using a 3cc syringe dispense 1.0 cc of blood into the TT or HNTT test tube. At the same time, depress the **START** key of the appropriate test well.

5b. *For citrated blood*: Withdraw 3 cc of citrated blood into a 3 cc syringe then dispense 1.0cc of blood into the TT or HNTT test tube. At the same time, depress the **START** key of the appropriate test well.

6. Immediately agitate the test tube vigorously from end to end ten times.
7. Insert the TT or HNTT test tube in the appropriate test well. Quickly rotate the tube clockwise. See appropriate instrument operator's manual for additional information.
8. At the indicator tone, record the test results.

PRODUCT USE WARNING

Note: *Observe universal precautions at all times.*

1. The blood specimen should be transferred using an appropriate transfer needle to pierce the stopper.
2. Always use a two-hand technique to transfer blood. One hand securely holds the tube while the second hand dispenses the blood specimen.
3. The TT and HNTT test tubes are made of glass. They can be broken or cracked if mishandled. Do not drop or toss tubes.
4. The TT and HNTT test tubes contain a material of biological origin (thrombin and protamine sulfate). Do not handle, aerosol, or ingest.
5. All used test tubes containing human derived blood should be discarded in approved biohazard containers.

PERFORMANCE CHARACTERISTICS

Normal Range

The TT and HNTT were evaluated in normal volunteer donors using fresh and citrated whole blood.

Test	Substrate	Mean (secs)	SD (secs)	Range* (secs)
HEMOCHRON TT	Fresh Blood	45.9	3.9	38-54
	Citrated Blood	49.3	3.7	41-57
HEMOCHRON HNTT	Fresh Blood	43.7	6.1	31-56
	Citrated Blood	47.0	5.4	36-58

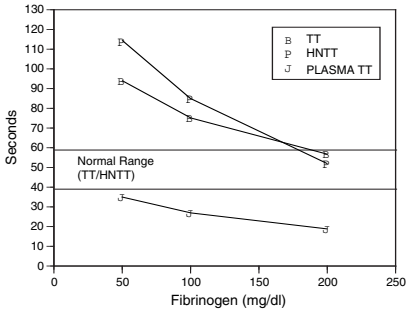
* Range = Mean ± 2 S.D

For optimal performance it is recommended that each institution establish its own normal range.

Fibrinogen Sensitivity

A standardized whole blood (SWB) sample was prepared with an assayed fibrinogen level of approximately 200 mg/dl. SWB with abnormal fibrinogen levels of 100 mg/dl and 50 mg/dl were also prepared. Comparison was made to a reference plasma thrombin time performed using a Fibrometer and bovine thrombin.

A dose response curve of sensitivity to fibrinogen was obtained for both test systems. The slope of the curve is indicative of the relative sensitivity of each assay.

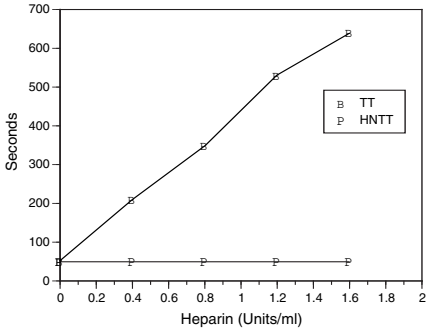


Heparin Sensitivity

Single Donor Sample

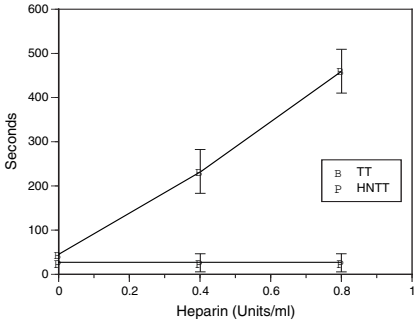
A heparin dose response curve was generated by the *in vitro* addition of heparin to freshly collected normal donor blood. Aliquots of the heparinized and unheparinized samples were assayed using the TT and HNTT.

The TT test showed a linear increase up to 1.6 heparin units/ml while the HNTT test remained unchanged over this range.



Response Range for Normal Donors

The above described procedure was performed for eight normal donors. The response range is shown at the right.



Response Range for Hospitalized Patients

Blood specimens were obtained from hospitalized patients. One group was receiving heparin and being monitored by the Activated Partial Thromboplastin Time test (APTT). A second group of patients was not receiving anticoagulant therapy.

Assay	Patient Group	
	NO HEPARIN	HEPARIN
HEMOCHRON TT	48.8 ± 2.2	143.0 ± 40.0
HEMOCHRON HNTT	47.7 ± 7.8	50.0 ± 6.2
APTT	27.3 ± 3.7	48.3 ± 13.2

All values are reported as seconds (mean ± 2 S.D.).

The TT is prolonged in the presence of heparin, which is confirmed by the elevated APTT values. The HNTT is unaffected by the presence of heparin.

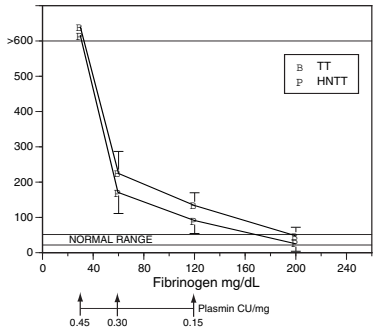
Sensitivity to Decreased Fibrinogen Levels Associated With Fibrinogenolysis

Since the TT and HNTT are sensitive to plasma fibrinogen levels, the test may conveniently be used to monitor the extent of fibrinogenolysis which occurs during thrombolytic therapy. Fibrinogenolysis was achieved in vitro by the addition of plasmin to normal donor blood. This results in a decrease of measurable fibrinogen which can be monitored by the TT and HNTT.

Freshly citrated blood samples were obtained from five donors. Plasmin was added to aliquots of the blood specimen at final concentrations of 0.15, 0.3, and 0.45 Casein Units (CU)/mg fibrinogen and incubated at 37°C for 45 minutes. The TT and HNTT were performed on these samples (A, below). In matched blood specimens, heparin (0.4 units/ml) was added following the plasmin incubation period and prior to performance of the TT and HNTT (B, below). Fibrinogen was quantified using a commercial assay. At any given plasmin concentration, the extent of fibrinogen digestion varies among individual donors. The values shown below represent mean values of 5 donor samples with similar (not identical) fibrinogen values.

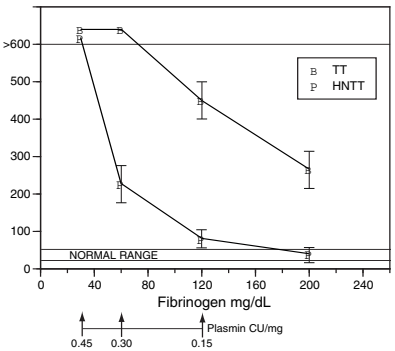
A. Plasmin Digestion, No Heparin

Plasma fibrinogen levels drop as the amount of added plasmin increases. The TT test and HNTT test increase as the fibrinogen level decreases.



B. Plasmin Digestion, Plus Heparin

In the presence of heparin the TT is prolonged from normal despite the presence of adequate fibrinogen. The TT continues to prolong as the fibrinogen level drops in response to plasmin digestion. The HNTT prolongs as the fibrinogen level decreases and is unaffected by the presence of heparin.



Interpretation of Results

TT N=39-53	HNTT N=33-58	Possible Causes
Normal	Normal	Normal FIB Function, No Circ. Heparin
Prolonged	Normal	Heparin
Prolonged	Prolonged	Abnormal Fibrinogen Function

1. A normal TT with a normal HNTT suggests that the fibrinogen level is normal and there is no circulating heparin detected.
2. A prolonged TT with a normal HNTT suggests the presence of heparin which may indicate the need for an additional small dose of protamine to achieve neutralization.
3. A prolonged TT and HNTT may suggest either a decrease of the fibrinogen level, requiring replacement therapy, or the presence of excessive heparin, requiring protamine.

LIMITATIONS

The TT and HNTT are affected by poor technique including sample collection and test procedure. Proper specimen/reagent mixing is required for precise and accurate testing. The following may affect results or be misleading in test interpretations:

1. Laboratory studies have shown that in the presence of normal plasma fibrinogen the accumulation of the degradation products of fibrinogen and fibrin (FDP's) do affect the resultant values. At excessive levels of FDP (400 ug/ml), alterations of results may be seen. Less excessive levels of FDP's in the presence of a reduced level of functional fibrinogen may also affect performance of the assay.
2. Test kits that have been improperly stored, affected by heat, or expired.
3. Test results which do not agree with expected values should be verified and, there after, evaluated by alternative diagnostic means.

QUALITY CONTROL

Routine quality control (QC) testing and tracking should be a part of a comprehensive quality assurance program. HEMOCHRON Whole Blood Coagulation System Quality Control products are available to make routine QC convenient and affordable.

Daily Instrument QC

At a minimum, all HEMOCHRON instruments should be quality controlled at two levels of performance, including both the normal and abnormal ranges, once every 8 hours of operation.

To assist in accomplishing daily QC, Electronic System Verification Tubes are available to provide multiple level (normal and abnormal) quality control checks on the instrument. Electronic System Verification should be performed once every 8 hours during which the instrument is utilized. This will ensure proper instrument operation.

QC of HEMOCHRON Test Tubes

Each box of HEMOCHRON test tubes should be validated at least once, prior to use. This can be accomplished by using the appropriate HEMOCHRON Liquid Quality Control. Acceptable performance ranges for the test tubes are included in the HEMOCHRON Quality Control Product Kit. After each individual box of test tubes has been verified, the "Performance Verified" table provided on the side panel of each test tube box should be completed. This box is now "IN CONTROL" and will not require further liquid control unless a shift in clinical results is experienced.

***NOTE:** If multiple boxes are received within the same shipment, it is recommended to validate each box upon opening, prior to use.*

REFERENCES

1. Shaw ST:Assays for fibrinogen and its derivatives. CRC Crit Rev Clin Lab Sci 8:145, 1977.
2. Cowen J, Khan M, Vargo J and Joist H: An improved method for evaluation of blood coagulation in heparinized blood. AJ Clin Path 75:60, 1981.
3. Yang V, Lindhart R, Bernstein H, Cooney C and Langer R: Purification and characterization of heparinase from flavobacteruim heparinum. J Biol Chem 260:1849, 1985.
4. Cumming A, Jones G. Wensley R and Cundall RB: In vitro neutralization of heparin in plasma prior to the activated partial thromboplastin time test: An assessment of four heparin antagonists and two anion exchange resins. Thombos Res 41:43, 1986.
5. Hurt R, Perkins H, Osborn J and Gerbode F: The neutralization of heparin by protamine in extracorporeal circulation. J Thorac Surg 32:612, 1956.
6. Shafer K, Santoro S, Sobel B and Jaffe A: Monitoring activity of fibrinolytic agents. Am J Med 76:879, 1984.
7. Marder V, Martin S, Francis C and Colman R: Consumptive thrombohemmorrhagic disorders, In, Hemostasis and Thrombosis, Basic Principles and Clinical Practice, ed R Colman, J.B. Lippincott, Phila, PA, p975-1015, 1987.
8. Comerota A, and Kerr T: Physiologic and therapeutic fibrinolysis, Sem Vasc Surg 1:239, 1988.
9. Mueller H, Roa A, Forman S and the TIMI Investigators: Thrombolysis in myocardial infarction (TIMI): Comparative studies of coronary reperfusion and systemic fibrinogenolysis with two forms of recombinant tissue-type plasminogen activator. J Am Coll Cardiol 10:479, 1987.
10. Bovill E, Stung D, Tracy R, Collen D, Robertson T, Terin M and Mann K:Dose response relationship of RT-PA infusion to induction of systemic fibrin(ogen) olysis in the thrombolysis in myocardial infarction (TIMI) Trial. Blood 70:367a, 1987 (Abst).
11. Francis C and Marder V: Physiologic reregulation and pathologic disorders of fibrinolysis, In, Hemostasis and Thrombosis, Basic Principles and Clinical Practice, ed R Colman, J.B. Lippincott, Phila, PA, p358-379, 1987.
12. Bell W and Meek A: Guidelines for the use of thrombolytic agents, N Engl J Med 301:1266, 1979.
13. Rubin R: Fibrinolysis and its current usage. Clinical Therapeutics 5:211, 1983.
14. Gold H, Leinbach R, Garabedian S, Yasuda T, Johns J, Grossbard E, Palacios I and Collen D: Acute coronary reocclusion after thrombolysis with recombinant human tissue-type plasminogen activator: Prevention by maintenance infusion. Circulation 73:347, 1986.
15. LaDuca F, Zucker M and Walker C: Assesing heparin neutralization following cardiac surgery: Sensitivity of thromin time-based assay versus protamine titration methods. Perfusion 14:181, 1999.

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HEMOCHRON®

Whole Blood Coagulation Systems

Thrombin Time (TT)/
Heparin Neutralized Thrombin Time (HNTT)

Package Insert

INTENDED USE

The HEMOCHRON Thrombin Time (TT) is a screening assay for the evaluation of the level of circulating fibrinogen and for the detection of heparin. The HEMOCHRON Heparin Neutralized Thrombin Time (HNTT) is designed to be solely indicative of the level of circulating fibrinogen and is unaffected by the presence of heparin. The TT and HNTT tests can be used with either fresh or citrated whole blood. The tests are intended solely for use with HEMOCHRON models *Response*, 8000, 801 or 401.

For *in vitro* Diagnostic Use

SUMMARY AND EXPLANATION

The TT and HNTT are whole blood tests of blood coagulation. They are useful in quantifying circulating fibrinogen, detecting the presence of heparin, diagnosing intravascular coagulation, and assessing thrombolytic therapy.

By performing simultaneous TT and HNTT assays, it is possible to differentiate hypocoagulability attributable to fibrinogen depletion from that caused by heparin anticoagulation. Therapeutic intervention may then be planned accordingly.

In humans, thrombin is generated from the precursor protein, prothrombin. Thrombin is a potent enzyme which readily converts soluble fibrinogen to insoluble fibrin. Fibrin deposition at the site of vascular injury is critical to clot formation and the control of bleeding. By the addition of thrombin reagent to a blood specimen, one is able to simulate in the laboratory the basic physiologic mechanism by which a fibrin clot is formed.

The thrombin time is a simple and extremely valuable general coagulation screening assay. The time required for the formation of a fibrin clot provides information regarding the level of functional fibrinogen and is prolonged in the presence of decreased fibrinogen (hypofibrinogenemia) or non-functional fibrinogen (dysfibrinogenemia).[1]

The thrombin time is commonly employed to detect suspected heparin anticoagulation[6] and to quantify circulating fibrinogen[1]. Measurement of the fibrinogen level is useful in the diagnosis of consumptive coagulopathy or intravascular coagulation[7] and the monitoring of thrombolytic therapy[6].

In addition to sensitivity to fibrinogen level, the thrombin time is sensitive to the presence of heparin. Heparin prolongs the thrombin time through neutralization of the thrombin found in the test tube. Since heparin is frequently encountered in the clinical setting, a variety of techniques have been used to neutralize the anticoagulant effect of heparin[2-5]. The most common of these is protamine sulfate[5]. The results of a coagulation assay, such as the thrombin time, performed on a blood specimen in which heparin has been neutralized, reflect the hemostatic condition of the patient. Such an assay may be referred to as the heparin neutralized thrombin time (HNTT).

Thrombolytic agents are given to patients with acute thrombotic episodes including myocardial infarction, deep vein thrombosis and pulmonary embolism[8]. These agents, which include streptokinase (SK), urokinase (UK), recombinant Tissue Plasminogen Activator (rTPA) and anistreplase (APSAC) activate the fibrinolytic system by the conversion of plasminogen to plasmin. Under normal hemostatic conditions, plasmin digests localized fibrin clots. However, as plasmin levels increase dramatically, such as occurs during thrombolytic therapy, fibrinogen is also systemically degraded[8, 11]. This results in a decrease of circulating fibrinogen and an increase of the Fibrinogen Degradation Products (FDP's)[11] during SK[6, 8], UK[6, 8] or rTPA[9, 10] therapy.

A systemic fibrinogen depletion associated with intravascular coagulation or the use of thrombolytic agents is best monitored with the thrombin time test[1, 6, 12]. Since heparin is given sequentially or concomitantly in most thrombolytic protocols[6, 13, 14] a heparin neutralized thrombin time provides the means to monitor the level of circulating fibrinogen without heparin interference[15].