

T –Tek

Thrombin Time Test



INTENDED USE

The T-Tek thrombin time reagent is intended for use in the quantitative determination of Thrombin Time (TT) in citrated human plasma in the general patient population. T-Tek should be used in the clinical laboratory by qualified laboratory professionals. The test may be performed using manual methods or semi-automated or automated coagulation analyzers.

SUMMARY

The active coagulation enzyme, thrombin, acts on soluble fibrinogen to convert it to insoluble fibrin. This reaction is manifested by the appearance of a visible fibrin clot (1).

Any substance that interferes with this reaction, i.e. heparin or fibrin degradation products, may produce a prolonged thrombin clotting time. Low plasma fibrinogen levels as well as abnormal fibrinogen molecules seen in severe liver disease or as congenital abnormalities will also prolong the clotting time.

PRINCIPLE

Thrombin solution is added to citrated plasma pre-warmed to 37°C and the time taken to detect the clot end point is noted.

REAGENTS

Warning: FOR IN VITRO DIAGNOSTIC USE ONLY

Warning: POTENTIAL BIOHAZARD. The plasma used to prepare the thrombin reagent is of human origin and should be considered potentially infectious. The plasma was tested and found negative for Hepatitis B antigen (HBsAg) and antibodies to HIV and HCV by FDA licensed tests.

1. T-Tek Thrombin Reagent

Ingredients: Each vial of reagent contains a lyophilized preparation of human thrombin of approximately 10 NIH units per mL plus added stabilizers and buffers.

Preparation for Use: Reconstitute each vial with 1 mL of distilled water as indicated on the vial. Swirl gently; do not shake. Allow to stand at room temperature for 10 minutes at room temperature before use.

Storage and Stability: The lyophilized product is stable until the expiration date printed on the vial, when stored at 2-8°C. After reconstitution, the reagent is stable at room temperature for up to 8 hours.

INSTRUMENTS OR TECHNIQUES

The thrombin time assay may be performed by accepted manual methods, or by using optical or electromechanical coagulation analyzers.

SPECIMEN COLLECTION AND HANDLING

Specimen: Plasma obtained from whole blood anti-coagulated with 0.1 M sodium citrate.

Specimen Collection: Nine parts of freshly collected whole blood should be immediately added to one part citrate anticoagulant and mixed thoroughly.

Specimen Preparation: Centrifuge the whole blood specimen at 2500 x g for 15 minutes (NCCLS H21-A2, 1991). Immediately separate the plasma from the red blood cells using a plastic pipette (if necessary) and place it in a plastic test tube.

Storage and Stability: Before and during testing the plasma sample should be maintained in plastic test tubes at 20 ± 5 °C to insure further stability. If testing is delayed for more than 8 hours, plasma may be stored at -20°C or below for up to one month. Frozen samples should be thawed rapidly at 37°C before testing.

TEST PROCEDURE

Reagents provided:

10 x 1 mL vials T-Tek Thrombin reagent

Reagents and equipment required but not provided:

1. Purified water for reconstitution
2. Stopwatch or timing device
3. Reagent cups or 12 X 75 mm plastic test tubes
4. Coagulation analyzer or 37°C water bath
5. Variable volume pipettes (100 & 200 uL)
6. Control Plasmas

Additional equipment and supplies available from r² Diagnostics:

PlasmaCon N
PlasmaCon L-1
PlasmaConL-2

STEP-BY-STEP METHOD

The following is the manual method. Please refer to the User Manual for instructions if using an automated instrument.

All test tubes and pipette tips should be plastic.

1. Reconstitute reagent as described.
2. Reconstitute lyophilized plasma controls as directed. Collect and prepare the plasma sample specimen according to the directions outlined in the SPECIMEN COLLECTION AND PREPARATION section.
3. To an instrument cuvette or test tube:
 - Add 200uL control or patient plasma
 - Incubate for 2 minutes at 37°C
 - Add 200uL Thrombin reagent
 - Start stopwatch
 - Record clotting time from timer

RESULTS

The clot times should fall within the laboratory's normal range. If clot times are prolonged, the fibrinogen level or activity is low or thrombin inhibitors may be present.

QUALITY CONTROL

All coagulation testing should include adequate quality control testing to verify instrument and reagent performance. No patient results should be reported unless the QC controls are within their reference ranges.

LIMITATIONS

Significant levels of heparin, Fibrin(ogen) Degradation Products (FDP), or Direct Thrombin Inhibitors (DTI such as hirudin) in the patient plasma may give a prolonged time. Samples that are hemolyzed, icteric, or lipemic may also interfere with the assay especially on photo-optical instruments.

EXPECTED VALUES

The normal values obtained are instrument and technique dependent, and each laboratory should determine its own reference range. Patient results should fall within the reference range for that laboratory. The ranges supplied below were obtained from 120 normal donors.

Normal range (N = 120): 13-15 seconds (photo-optical)
16-18 seconds (mechanical)

PERFORMANCE CHARACTERISTICS

1. Precision

Precision studies were performed to establish Within Run and Between Run CV's for normal and abnormal controls. Assays were performed using photo-optical and mechanical coagulation analyzers. Combined results are shown below.

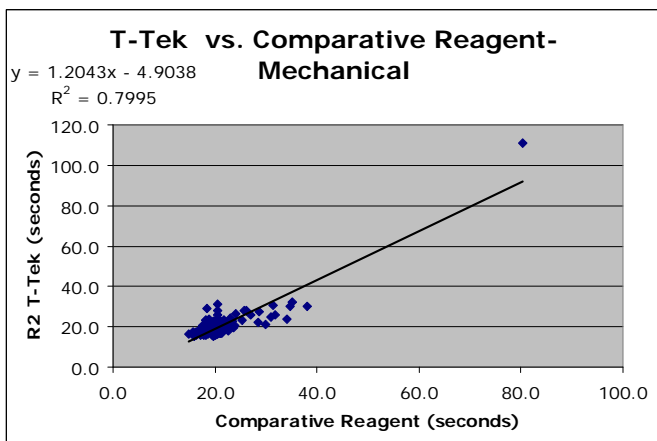
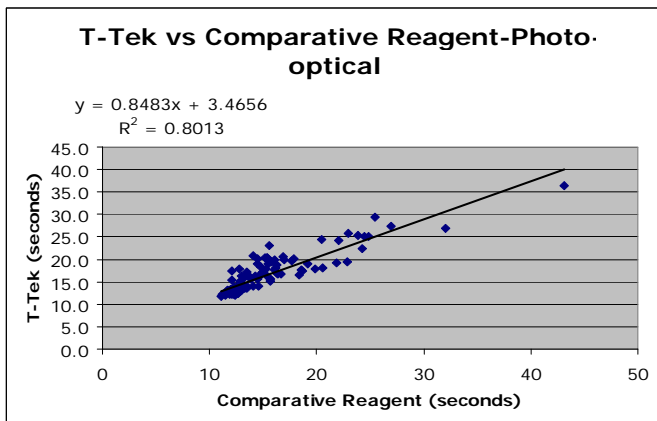
<i>Normal</i>	<i>Within Run</i>	<i>Between Run</i>
n	40	20
Mean	16.4 seconds	16.9 seconds
SD	0.35 seconds	0.37 seconds
CV	2.25%	2.33%

<i>Abnormal</i>	<i>Within Run</i>	<i>Between Run</i>
n	40	20
Mean	20.5 seconds	20.4 seconds
SD	0.6 seconds	0.34 seconds
CV	3.0%	1.72%

2. Comparison

A comparison study was done using the T-Tek assay and a comparative method on at least 125 normal and abnormal frozen clinical samples using two different coagulation analyzer types. The linear regression equations and the coefficient of determination (r^2) were as follows:

1. Clauss, A., Acta Haemat. 17:237-246, 1957



REFERENCES

