# Tiempo de Trombina

**For thrombin time determination**

## SUMMARY

Thrombin time forms part of the coagulometric screening tests used to detect the cause of a hemostatic disorder.
Whenever a trauma or vascular injury is produced, thrombin cleaves the soluble fibrinogen in fibrin monomers that spontaneously polymerize forming an insoluble fibrin clot. The thrombin time evaluates this last stage of the coagulation process, i.e. the conversion of the fibrinogen to fibrin. Therefore, the anomaly in the fibrinogen function, the presence of substances interfering in the thrombin influence over the fibrinogen (heparin, hirudin) or the ones blocking the polymerization of fibrin monomers (FPD/fpd, paraproteins) prolong the Thrombin Time test. This determination does not detect factor XIII deficiencies.
Thus, any alteration in the Thrombin Time may be due to qualitative or quantitative fibrinogen abnormalities, increased fibrinolysis, therapies using anticoagulants, fibrinolytic therapies, etc.

## PRINCIPLE

Thrombin converts into fibrin the fibrinogen contained in the plasma sample, forming a clot. The time to clot formation is measured.

## PROVIDED REAGENTS

**A. Reagent A:** vials containing freeze-dried bovine thrombin (8.0 UNIH/vial approximately) with buffer and stabilizers.

## NON-PROVIDED REAGENTS

Bidistilled or deionized water.

## INSTRUCTIONS FOR USE

Each Reagent A vial may be reconstituted in two different ways, according to the required analytical application:

| Reconstitution volume | Thrombin units |
|---|---|
| 2 ml | 4.0 UNIH/ml |
| 3 ml | 2.7 UNIH/ml |

Reconstitution: open the vial by slowly removing the rubber cap to avoid any loss of material.
Add the volume of bidistilled or deionized water indicated above.
Cap; let stand for 30 minutes and mix gently (without shaking) until obtaining a complete dissolution. Date.
After reconstitution and during use, it is recommended to store the Reagent A in its original vial.

## WARNINGS

The Reagent is for “in vitro” diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

## STABILITY AND STORAGE INSTRUCTIONS

**Reagent A:** stable at 2-10ºC until the expiration date shown on the box.
**Reconstituted Reagent A:** stable for up to 7 days at 2-10ºC. For longer periods of time, samples can be frozen at -20ºC for up to 30 days in their original vial or in fractionated aliquots in Eppendorf or plastic tubes. Thaw samples rapidly at 37ºC. Do not re-freeze. Avoid prolonged warming.
To optimize the reagent’s stability it is recommended to cap it after use and store at 2-10ºC in its original vial.

## SAMPLE

Plasma
**a) Collection:** carefully collect blood, avoiding foam formation and place it in a collection tube with anticoagulant in an exact ratio of 9+1 (e.g. 4.5 ml blood + 0.5 ml anticoagulant). Mix gently. Centrifuge and separate plasma before 30 minutes. Collection should be performed using plastic syringes.
**b) Additives:** to obtain plasma use Wiener lab.’s **Anticoagulant TP** or else 3.8 or 3.2% sodium citrate. Do not use EDTA or heparin.
**c) Known interfering substances:** if reagent is manually used, no interferences are observed with bilirubin up to 20 mg/dl, triglycerides up to 2000 mg/dl, or hemoglobin up to 250 mg/dl. See Young, D.S. in References for effect of drugs on the present method.

## REQUIRED MATERIAL (non-provided)

- Hemolysis tubes.
- Micropipettes for measuring the stated volumes.
- Water bath at 37ºC.
- Stopwatch.
- Light source for clot observation.

## PROCEDURE

Bring the reagent to room temperature before use.
1- In prewarmed hemolysis tubes add 0.2 ml of each plasma and incubate at 37ºC for 2 minutes.
2- Rapidly add 0.2 ml Reagent A and record the time required for clot formation.
3- Repeat the test and calculate the mean result for each sample.

## INTERPRETATION OF RESULTS

Results are reported in seconds.
In case the difference between replicates of the same sample were more than 5%, it is recommended to repeat procedure discarding previous values.

## QUALITY CONTROL METHOD

Wiener lab.'s Plasma Control normal - patológico.

## REFERENCE VALUES

Values observed in normal patients using the manual method are between:

13 - 17 sec for 4.0 UNIH/ml
17 - 21 sec for 2.7 UNIH/ml

These values serve only as a reference, since the coagulation time is influenced by different variables, such as collection and storage of the sample, techniques and instrumentation used, etc. Therefore, each laboratory should establish its own reference intervals, adjusting the variables influencing the assay.

## PROCEDURE LIMITATIONS

- See Known Interfering Substances under SAMPLE.
- Failure in the reconstitution of the reagent may cause erroneous results.
- Sample collection:
  Samples should be placed in plastic or siliconized glass tubes.
  Icteric, lipemic or hemolyzed samples may yield erroneous results when using photometric detection of the clot.
  It is important to maintain the anticoagulant and blood relation as well as the citrate concentration used.
- Laboratory technique:

The assay should be performed at 37ºC and the tubes should be thoroughly clean and dry.
After performing the assay in an autoanalyzer, adequate cleaning measures should be adopted to prevent subsequent contamination with the reagent's thrombin.

## PERFORMANCE

**Reproducibility:** testing replicates of the same samples on the same day, the following values were obtained:

| Thrombin Time | 4.0 UNIH/ml | | 2.7 UNIH/ml | |
|---|---|---|---|---|
| | Level 1 | Level 2 | Level 1 | Level 2 |
| n | 20 | 20 | 20 | 20 |
| $\overline{X}$ (sec) | 15.5 | 17.4 | 17.5 | 19.3 |
| S.D. (sec) | 0.28 | 0.29 | 0.24 | 0.34 |
| C.V. (%) | 1.79 | 1.67 | 1.37 | 1.76 |

## PARAMETERS FOR AUTOANALYZERS

The Reagent can be manually used as well as with mechanical and photo-optical reading coagulation systems. For semi-automatic and automatic instruments please refer to the instructions given by the instrument manufacturer.

## WIENER LAB. PROVIDES

- Kit for 60 or 90 determinations (Cat. Nº 1705009):
  Reagent A: 6 vials (2 or 3 ml)

## REFERENCES


- Harrison, R. - Am. J. Clin. Pathol. 89/1:87 (1988).
- Penner, J. - Am. J. Clin. Pathol. 61:645 (1974).
- Glynn, M.F.X. - Am. J. Clin. Pathol. 71:397 (1979).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

# Symbols

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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