Prothrombin Time

plus

Calcium thromboplastin for one stage prothrombin time determination

SUMMARY

Prothrombin Time or Quick Time is the screening test of greatest clinical importance in the evaluation of coagulation extrinsic pathway disorders. Due to its sensitivity to qualitative and quantitative extrinsic and common path factor alterations, it may be used for:
- Oral anticoagulant therapy monitoring, due to its sensitivity to dependent vitamin K factors (II, VII and X).
- Simple or combined factor deficiencies detection, due to hereditary or acquired alterations (liver diseases, vitamin K deficiency, etc.).
- Pre-surgical studies.
- Specific determination of factor II, V, VII and X activity.

PRINCIPLE

The method is based on the measurement of fibrin clot formation time, by adding calcium thromboplastin to a citrated plasma.

PROVIDED REAGENTS

A. Reagent A: rabbit brain thromboplastin with 10 mM calcium chloride final concentration.

NON-PROVIDED REAGENTS

- Saline solution.
- **Coagulation Calibrator** from Wiener lab.

INSTRUCTIONS FOR USE

Reagent A: ready to use. Homogenize each time it is used.

WARNINGS

The reagent is for "in vitro" diagnostic use.
All patient samples should be handled as if they were capable of transmitting infection.
Use the reagents keeping the usual work precautions in the clinical chemistry laboratory.
All reagents and samples must be discarded according to the local regulations in force.

ESTABILITY AND STORAGE INSTRUCTIONS

Reagent A: stable in refrigerator (2-10ºC) until the expiration date indicated on the box. Do not freeze.

SAMPLE

Citrated plasma.
a) Collection: collect blood carefully (avoiding stasis or trauma) and place in a tube with anticoagulant in an exact 9 + 1 ratio (example: 4.5 ml blood + 0.5 ml anticoagulant). Mix gently. Centrifuge for 15 minutes at 2500 g and separate plasma before 30 minutes. It is advisable to perform collection with plastic syringes.
b) Additives: to obtain plasma use Anticoagulante PT from Wiener lab, or 130 mmol/l (3.8%) or 109 mmol/l (3.2%) sodium citrate.
c) Known interfering substances:
- EDTA or heparin should not be used to obtain plasma.
- Contamination, visible or not, may cause falsely prolonged periods.
- Hemolysis and visible lipemias obstruct photooptical measurement of results.

Refer to Young's bibliography for the effects of drugs in the present method.
d) Stability and storage instructions: plasma should be kept at room temperature until the test is performed (do not store at 2-10ºC). This period should not be prolonged for more than 4 hours. If not processed, plasma may be frozen for up to 2 weeks at -20ºC. In this case, sample should be frozen immediately and thawed rapidly at 37ºC, not prolonging this period by more than 10 minutes.

NON-PROVIDED REQUIRED MATERIAL

- Khan or hemolysis tubes.
- Pipettes and micropipettes for measuring stated volumes.
- Water bath at 37 ± 1ºC or semiautomatic or automatic coagulometer.
- Chronometer.

PROCEDURE

1- Preheat Reagent A at 37ºC (no more than 20 minutes)
2- In a tube preheated at 37ºC, place 100 ul sample. Incubate for 1 minute in a water bath at 37ºC.
3- Trigger timer with the addition of 200 ul pre-heated reagent A. Prior to the estimated coagulation time, remove the tube from bath, gently sliding the liquid content from the bottom to the middle of the tube and stop the stopwatch at the time of clot appearance.
4- Record clot formation time.
5- Repeat the determination and average the result for each sample. If the difference between the replicates is greater than 5%, it is advisable to repeat the procedure.

INTERPRERATION OF RESULTS

The results can be expressed in different ways:
1- Prothrombin time (PT) in seconds.

2- Prothrombinic Activity percentage (% PT): prepare a calibration curve for each reagent batch from a calibration plasma (Coagulation Calibrator from Wiener lab.) or from a pool of fresh plasmas (at least 20 plasmas from healthy individuals) with PT between 90-110%) using saline solution as diluent:

Prothrombinic Activity Percentage Curve

Tube N°	SF (ml)	Calibrator (ml)	Activity (%)	Activity (%)
1	-	1.0	100*	A x 1
2	0.3	0.7	70	A x 0.70
3	0.5	0.5	50	A x 0.50
4	0.7	0.3	30	A x 0.30
5	0.8	0.2	20	A x 0.20
6	0.9	0.1	10	A x 0.10

* When the **Coagulation Calibrator** kit is used, the activity value stated in the kit´s insert (A) is considered 100% and for the rest of the dilutions that value is multiplied by the dilution factor used.
Prepare the dilutions immediately before drawing the curve. Determine the PT of each dilution in duplicate and draw on graph paper PT means according to activity %; or on log-log paper to linearize the calibration curve.
Perform a calibration curve each time a reagent lot is changed or when the quality control indicates it. For a semiautomatic coagulometer, process the dilutions of the curve and enter the PT means obtained in the calibration method of the instrument´s PT. With the calibration loaded in the instrument, the PT (sec) of each sample will be interpolated automatically by the instrument software, obtaining in each case the corresponding prothrombinic activity. In the case of automatic coagulometers, the dilutions are performed by the instrument and, based on the calibration curve obtained, the samples tested are directly informed with their prothrombin activity %.

3- According to the World Health Organization (WHO), PT results (seconds) of patients under treatment with oral anticoagulants in stable phase, should be expressed in INR (International Normative Ratio) to be independent of the measurement system (Reagent/Instrument) used, using the following formula:

INR: $(\text{patient´s PT}/\text{MNPT})^{ISI}$ where:

Patient´s PT: average patient prothrombin time in seconds.
MNPT: geometric mean of the PT of a normal adult population. It is calculated for each batch of reagent with at least 20 samples of fresh plasmas from healthy adult individuals.
ISI: International Sensitivity Index. It is obtained for each measurement system: reagent/instrument, according to WHO recommendations.

For a semiautomatic and automatic coagulometer, enter the ISI and MNPT method values of the specific system: reagent/instrument. Thus, the samples tested will be informed directly with their corresponding INR.

QUALITY CONTROL METHOD

Plasma Control normal - patológico from Wiener lab.
Coagulation Control N - Coagulation Control P from Wiener lab.
Controls are processed in the same way as samples.

REFERENCE VALUES

70 - 120%
10 -14 seconds (indicative range - depends on the measurement system)
It is recommended that each laboratory establish its own reference intervals, within its patient population.
The therapeutic INR ranges may vary according to the indications of oral anticoagulant therapy.

PROCEDURE LIMITATIONS

See Known Interfering Substances in SAMPLE. Any modification in collection, blood/anticoagulant relation, processing and storage of the sample will cause an erroneous result in the determination.
Hemolysed and/or coagulated samples should be discarded. Store plasma samples at room temperature to avoid activation by low temperature. See SAMPLE. The presence of lupus anticoagulant or thrombin inhibitors can affect the determination by altering results.
Pre-incubation of the reagent at 37ºC should not exceed 20 minutes. A new calibration is required for each batch of reagents and instrument used.

PERFORMANCE

a) Imprecisión: evaluada a través del protocolo EP15A2 del CLSI procesando controles.

Intra-assay Precision

Sample	Level	C.V.
Plasma Control normal	13,5 sec	1,45%
Plasma Control patológico	49,9 sec	1,57%

Total Precision

Sample	Level	C.V.
Plasma Control normal	13,5 sec	1,58%
Plasma Control patológico	49,9 sec	1,66%

b) Sensitivity to heparin:
Prothrombin Time plus is not sensitive up to 1.5 U/ml heparin
c) Analytical sensitivity:
Prothrombin Time plus presents an ISI range (International Sensitivity Index) lower than 1.2.

WIENER LAB PROVIDES

- 10 x 4 ml (Cat. No. 1705027)
- 10 x 10 ml (Cat. No. 1705028)

REFERENCES

- Quick, A.J. - “Fisiología y Patología de la Hemostasis” - Ed. El Ateneo, Buenos Aires (1952).

- Araldi, H.T., et al. - “Primer Reactivo Nacional Argentino de Referencia de Tromboplastina de Cerebro Humano” - Acta Bioquím. Clín. Latinoam. XVI/1:131 (1982).
- Comité de Expertos de la O.M.S. en Patrones Biológicos - Inf. Nº 28: Normalización de la Vigilancia del Tratamiento Anticoagulante (oral) - Serv. Inf. Tec. Nº 610:49-56 (1977).
- Comité de Expertos de la O.M.S. en Patrones Biológicos - Inf. Nº 31: Requerimientos para Tromboplastinas y Plasmas usados en la terapia anticoagulante oral - Serv. Inf. Téc. Nº 658:202-223 (1981).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests". AACC Press, 4th ed., 2001.

SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents 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	Contains 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:
	Harmful
	Corrosive / Caustic
	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
http://www.wiener-lab.com.ar
Tech. Dir.: Viviana E. Cétola
Biochemist
A.N.M.A.T. Registered product
PM-1102-174

Wiener lab.

2000 Rosario - Argentina