Soluplastin

Calcium thromboplastin for the determination of one-stage Prothrombin Time

SUMMARY

Prothrombin time or Quick Time is a screening test of major clinical importance in the evaluation of disorders of the extrinsic coagulation pathway. Its sensitivity to qualitative and quantitative alterations of factors of the extrinsic and common pathway allows it to be used in:

- Detection of simple or combined factor deficiencies, due to hereditary or acquired alterations (hepatopathies, vitamin K deficiency, etc.).
- Pre-surgical studies.
- Specific determination of factor II, V, VII and X activity.
- Monitoring of therapy with oral anticoagulants, due to its sensitivity to vitamin K dependent factors (II, VII and X).

PRINCIPLE

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

PROVIDED REAGENTS

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

NON-PROVIDED REAGENTS

- Bidistilled or deionized water.
- Saline solution.
- **Coagulation Calibrator** from Wiener lab.

INSTRUCTIONS FOR USE

- Open a vial removing the metal precinct and slowly pulling out the rubber stopper to avoid any loss of material.
- Add the bidistilled or deionized water volume indicated on the label. Cover, let stand for 30 minutes at room temperature and then homogenize the solution by gentle agitation before use.
- Homogenize each time before use.

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 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: is stable in refrigerator (2-10ºC) until the expiration date shown on the box.

Reconstituted Reagent A: in refrigerator (2-10ºC) is stable for 5 days from its reconstitution. Do not freeze.

SAMPLE

Citrated plasma

a) Collection: obtain blood carefully (avoiding stasis ortrauma) and place it in a tube with anticoagulant in an exact 9 + 1 proportion (e.g.: 4.5 ml blood + 0.5 ml anticoagulant). Mix gently. Centrifuge for 15 minutes at 2500g and separate the plasma before 30 minutes. Collection using plastic syringes is recommended.

b) Additives: to obtain plasma, Wiener lab’s **Anticoagulante TP**, or 130 mmol/l (3,8%) or 109 mmol/l (3,2%) sodium citrate should be used.

c) Known interfering substances:

- Do not use EDTA or heparin for plasma collection.
- Contaminations, visible or not, yield falsely extended times;
- Visible hemolysis and lipemias interfere with the photooptical reading of the results.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: plasma should be kept at room temperature until tested (do not store at 2-10 ºC). This period should not be extended for more than 4 hours. If plasma is not processed within this period, it must be frozen for up to 2 weeks at -20ºC. This procedure should be done promptly, as well as the thawing at 37ºC, not extending this period longer than 10 minutes.

REQUIRED MATERIAL (non-provided)

- Khan or hemolysis tubes
- Micropipettes and pipettes for measuring the stated volumes
- Water bath at 37ºC
- Stopwatch

PROCEDURE

1- Preheat Reagent A at 37ºC (no longer than 20 minutes).

2- In a preheated tube at 37ºC, place 100 µl sample. Incubate for 1 minute in water bath at 37ºC.

3- Start the stopwatch with the addition of 200 µl preheated Reagent A. Before the estimated coagulation time, remove the tube from the water bath, gently slide the liquid content from the bottom to the middle of the tube and stop the stopwatch at clot formation.

4- Record the clot formation time.

5- Repeat the determination and average the result for each sample. If the difference between replicates is greater than 5%, it is advisable to repeat the procedure.

INTERPRETATION OF RESULTS
The results can be expressed in different ways:

1- Prothrombin Time (PT) in seconds.

2- Prothrombin Activity Percentage (%PT)

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

Curve of Prothrombin Activity Percentage

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

The preparation of the dilutions should be done immediately before the curve.
Determine the PT of each dilution in duplicate and plot on graph paper the PT means in terms of activity % or on log-log paper to linearize the calibration curve.

* When the **Coagulation Calibrator** kit is used, the activity value given in the kit's insert (A) is considered to be 100% and for the rest of the dilutions the said value is multiplied by the dilution factor.

The calibration curve must be performed each time the reagent batch is changed or when the quality control indicates so.
For a semi-automatic coagulometer, process the dilutions of the curve and enter the PT averages obtained in the calibration method of the instrument PT. With the calibration loaded in the instrument, the PT (sec) of each sample will be automatically interpolated by the instrument software, obtaining in each case the corresponding prothrombin activity.
In the case of an automatic coagulometer, the dilutions are performed by the instrument from the calibration curve obtained, the samples tested are reported directly with their prothrombin activity %.

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

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

where:

Patient PT: mean of the patient's Prothrombin Time in seconds.
MNPT: PT geometric mean of the adult normal population. It is calculated for each batch of reagent with at least 20 samples of fresh plasmas from healthy adult subjects.
ISI: International sensitivity index. It is obtained for each measurement system: reagent/instrument, using WHO recommendations.

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

QUALITY CONTROL METHOD
Plasma Control Normal - Patológico from Wiener lab.
Coagulation Control N - Coagulation Control P from Wiener lab.
The controls are processed in the same way as the samples.

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

PROCEDURE LIMITATIONS
See Known interfering substances under SAMPLE.
Any modification in the collection, blood/anticoagulant ratio, processing and storage of the sample will cause an erroneous result in the determination.
Hemolyzed and / or coagulated samples should be discarded.
Store plasma samples at room temperature to avoid low temperature activation. See SAMPLE.
The presence of lupus anticoagulant or thrombin inhibitors may affect the determination by altering the results.
The preincubation of the reagent at 37°C should not exceed 20 minutes. On the other hand, it is recommended to store the reconstituted reagent in the refrigerator when not in use.
Avoid leaving the reagent at room temperature for extended periods of time.
A new calibration is required for each batch of reagents and instrument used.

PERFORMANCE
a) Reproducibility: precision studies were performed following the guidelines contained in NCCLS (National Committee on Clinical Laboratory Standards) EP5-A document.

Intra-assay precision

Sample	Level	S.D.	C.V.
Normal Control Plasma	12.5 sec	± 0.1 sec	1.0%
Pathological Control Plasma	31.7 sec	± 0.4 sec	1.4%
Pool of normal plasmas	11.2 sec	± 0.1 sec	1.3%
Pool of pathological plasmas	15.4 sec	± 0.2 sec	1.3%

Total precision

Sample	Level	S.D.	C.V.
Normal Control Plasma	12.5 sec	± 0.4 sec	2.9%
Pathological Control Plasma	31.7 sec	± 1.0 sec	3.2%
Pool of normal plasmas	11.2 sec	± 0.3 sec	3.0%
Pool of pathological plasmas	15.4 sec	± 0.4 sec	2.4%

b) Correlation: the results of INR from 105 healthy patients in a stable treatment phase with oral anticoagulants obtained with **Soluplastin** and a commercial kit of similar principle were compared, and the following correlation parameters were obtained:
$y = 0.9562 x + 0.1087$ ($R^2 = 0.9825$)

WIENER LAB. PROVIDES
- Kit for 100 tests (10 x 2 ml) (Cat. 1705001).
- Kit for 200 tests (10 x 4 ml) (Cat. 1705005).
- Kit for 320 tests (8 x 8 ml) (Cat. 1705003).

REFERENCES

- Quick, A.J. - “Fisiología y Patología de la Hemostasis” - Ed. El Ateneo, Buenos Aires (1952).
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- 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).
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- Suñer Casadevall, F. - “Nuevas Normas Internacionales para la Expresión del Tiempo de Quick” - Análisis Clínicos X/40:240-245 (1985).
- 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
Dir. Téc.: Viviana E. Cétola
Biochemist
A.N.M.A.T. Registered product
PM-1102-148

Wiener lab.
2000 Rosario - Argentina