PT
Insert

Ref.: **504**

Prothrombin Time

Intended use . System for determination of prothrombin time (PT) and measurement of prothrombin complex factors (factors II, V, VII, and X).

Professional use.

[Only for in vitro diagnostic use.]

Test principle . Thromboplastin (tissue factor or factor III) triggers the extrinsic pathway of the coagulation cascade, generating a complex with factor VII that is calcium-dependent. Factor VII is turned into an active enzyme (factor VIIa) that acts on factor X, generating factor Xa. This factor, along with tissue factor phospholipids, factor Va, and calcium, originates the Prothrombin Activation Factor, which turns prothrombin into thrombin. Thrombin acts on fibrinogen, turning it into fibrin. The formation of fibrin is macroscopically perceived by the formation of clot. PT represents the time necessary to generate fibrin after the addition of thromboplastin, plasma, and calcium. The results are obtained through the comparison between the time to coagulation observed for sample plasmas and the time to coagulation observed for reference plasmas, which represent the measurement of prothrombin complex factors (factors II, V, VII, and X).

Summary . The PT - Labtest reagent contains thromboplastin extracted from rabbit brain, which is very sensitive to isolated or combined deficiencies of factors II, V, VII, and X, when compared to other thromboplastins form rabbit brain. Its high sensitivity to the presence of PIVKAs (Protein Induced Vitamin K Absence or Antagonism), with responses that are very close to human thromboplastins, increases the test confidence for the control of anticoagulant therapy.

An important feature of the Labtest's reagent is its traceability to the WHO Third International Reference Reagent for Thromboplastin (RBT/05) and to the International Sensitivity Index (ISI) established with the First International Reference Preparation for Human Thromboplastin (IRP 67/40). Therefore, the results obtained with PT are substantially equivalent to the ones that would be found if PT were measured using IRP 67/40, thus standardizing the results worldwide[1].

Methodology . Coagulometry - Quick[2]

Reagents

1. R 1 - Reagent 1 - Store at 2 - 8 ºC.
Liquid, ready for use. Contains rabbit brain extract ≥2.9%, sodium chloride 105-145.5 mM, sodium citrate 25-30 mM, isothiazolinone 0.1 - 0.35%. Check the ISI value on the reagent label. Do not freeze.

The PT - Ref. 504 unopened, when stored in right conditions, is stable until the expiration date.

After opened, the reagent is stable for 14 days between 2 - 8 ºC.

Store the reagent protected from light and avoid long-term exposure to ambient light.

The Reagent 1 must be heat in a container with lid to avoid its evaporation. The prolonged exposure to the heat and atmospheric air can compromise its performance.
The reagent must be open only in the time to get the volume to be use on the test.
The reagent's stability can be jeopardize by the use of a dirty and contaminated filter.

Since it is a suspension, the reagent can have solid sediments on the bottom of the bottle. The reagent must be carefully homogenize until all the sediments become resuspended and get the homogenous aspect. Wrong results can be found without the homogenization of the reagent.

Precautions and warnings

The ordinary safety cares must be applied handling the reagents.

Store the reagent protected from light and avoid long-term exposure to ambient light.

Materials required not provided

1. Pipets to measure samples and reagent.
2. Water bath kept at a constant temperature of 37 ºC.
3. Timer

Preanalytical influences . PT may be increased for people who use corticosteroids, oral contraceptives (failure in excreting bile salts), asparaginase, clofibrate, erythromycin, ethanol, tetracycline, heparin, and warfarin, as well as EDTA. PT may be reduced for people who use antihistaminics, butabarbital, phenobarbital, oral contraceptives (diminished response to oral anticoagulants), vitamin K, and caffeine.

Specimen collection and preparation

Use plasma samples collected in anhydrous trisodium citrate 109 mmol/L (3.2%).

Samples and reference plasma should not be diluted.

Must be create an Standard Operational Procedure (SOP) that includes appropriates procedures for collection, preparation and storing of the sample. Mistakes on pre-analytical phase can be bigger than the ones during the analytical phase.

Considering that the sample quality is fundamental to obtain accurate results, it is strongly recommended that you use the following procedure:

Labtest

1. Obtain blood through venous puncture, avoiding prolonged tourniquet application, hemolysis, bubbles, and aspiration of tissue fluid (factor III). The needle must penetrate the vein directly on the first attempt (nontraumatic venous puncture). The blood must flow freely, without the need to use too much strength to pull the plunger. Do not use samples that were difficult to obtain (traumatic venous puncture).

2. Collect the sample using plastic syringe and centrifuge it in plastic tubes. The use of non-siliconized glassware activates the coagulation factors and falsely reduces PT. After removing the needle, use the central portion of the sample, using the first and last portions for other tests.

3. If you are using vacuum blood collection systems, use plastic or siliconized glass tubes. When performing the collection for coagulation tests, draw two samples. Place the first sample in a tube without anticoagulant or in a tube containing citrate (blue cover), and discard it. The second sample must be placed in a tube containing citrate (blue cover) and it will be used for performing the tests. In case of multiple collections, the sample for coagulation tests must be obtained after the collection in tube without anticoagulant and before the collection in tube containing EDTA.

4. Mix 9 parts of blood with 1 part of citrate, or 3 mL of blood with 1 drop of Thrombstab (Labtest Ref. 45). Homogenize the tube 3 or 4 times by gentle inversion. Do not use oxalate, since factor V is very sensitive to this anticoagulant.

5. In patients who have hematocrit higher than 55%, the ratio blood volume/anticoagulant must be modified to ensure the accuracy of results. To calculate the volume of anticoagulant taking into account the hematocrit and blood volume, use the following equation:

Anticoagulant Volume (mL) = 0.00185 x blood volume (mL) x (100 - hematocrit)

Example

For a hematocrit of 60%, use 0.22 mL of citrate and complete the volume to 3.0 mL with blood. When using Thrombstab (Labtest Ref. 45), add 2 drops to 0.5 mL of water and use it in the ratio indicated by the calculation.

6. Centrifuge the sample in less than 1 hour after collection at 3000 rpm or 1500 g for 15 minutes. It is not necessary to remove the plasma from the tube. Keep the tube closed until test time to avoid changes in the sample pH, which may interfere in results.

7. Keep samples at 18 - 24 ºC and perform the PT test up to 4 hours after collection. Do not refrigerate plasma, as factor VII may be activated by the kallikrein system, yielding falsely diminished results. If fast freezing is a feasible option, plasma may be kept at - 20 ºC for 2 weeks or - 70 ºC for 6 months. We recommend that samples be stored as 0.5mL aliquots to avoid sample evaporation during storage. Use adequate vials to store frozen samples ("cryotubes"). Samples must be rapidly thawed at 37 ºC and tested immediately.

8. The presence of clot indicates that the sample must be rejected.

Blood sample must be considered as potentially infectious. Therefore, the bio-security standards must be use when handling it. We suggest apply the local, state or federal environmental protection standards for the disposal of the reagents.

Interference

Hemolytic, icteric, and lipemic samples can modify the results in an unforeseeable way.

Procedure[3]

The reagent can have solid sediments on the bottom of the bottle. The reagent must be carefully homogenize until all the sediments be resuspend and gets the homogenous aspect. Wrong results can be found without the homogenization of the reagent.

The reagent must be open only in the time to get the volume to be use on the test. **Store the reagent protected from light and avoid long-term exposure to ambient light.**

The reagent can be used for determining PT in automated and semi-automated systems. Follow the instructions indicated by their vendors.
The following procedure applies for manual technique.

1. Prepare the reference plasma by mixing citrate plasmas (pool) of at least 3 healthy individuals. Do not use plasma from people with liver disease, pregnant women, or women using oral contraceptives. Obtain the time of the reference plasma for every lot of PT. It is recommended as Laboratory Best Practices that a register be kept for PT lots and reference plasma times.

2. Perform the test in strictly clean glass tubes.

3. The water bath temperature must be at 36 - 38 ºC.

4. Incubate 0.1 mL of plasma (reference, control, or test) for at least 1 minute up to 10 minutes.

5. Add 0.2 mL of Reagent 1 (previously heated to 37 ºC), starting the timer simultaneously. Mix carefully and keep it in water bath for 9 seconds.

6. Get the tube, tilt it successively and observe the formation of a clot that prevents the liquid from moving. Stop the timer immediately and register the time.

Calculation . Prothrombin ratio (PR), International Normalized Ratio (INR), and Prothrombin Activity (A%).

The results can be obtained from the "Conversion Table in PR, INR and A%". Find in the "Reference Pool" line, where the coagulation times are shown in boldface, the closest value to the one obtained for the reference pool. Next, find in the matching column the coagulation time for the "Test Sample". The results for the sample, expressed as PR, INR, and A%, can be found on the same line as the time in seconds for the test sample, in the last three columns of the table.

PT and INR can also be calculated using the following equations:

$$PR = \frac{\text{Time in seconds of patient's plasma}}{\text{Time in seconds of reference pool}}$$

The use of PR enables the standardization of results, thus eliminating the variables introduced by sample collection and method execution.

$$INR = PR^{ISI}$$

Calibration and rastreability . PT was calibrated using the WHO Third International Reference Reagent for Thromboplastin from WHO (RBT/05) and has its International Sensitivity Index (ISI) established against the First International Reference Preparation for Human Thromboplastin (IRP 67/40).

Internal quality control . The testing center must keep an internal quality control program that clearly defines all applicable regulations, objectives, procedures, and criteria for quality specifications and tolerance limits, corrective actions and registration of activities. Control materials should be used to assess imprecision and calibration deviations. It is recommended that you use the CLIA specifications for total error[6].

For the internal quality control program, Labtest has Qualitrol Hemostasis 1 - Ref. 507, Qualitrol Hemostasis 2 - Ref. 508 and Qualitrol Hemostasis 1 + 2 - Ref. 509 for the test's control of Prothrombin Time, Parcial Actived Thrombplastin and Fibrinogen time.

The comparison between results obtained with PT and products from other vendors can only be performed using the INR.

Expected values . These values should be used only as orientation. Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Prothrombin Activity: higher than 70%. Values above 100% do not have clinical significance and should be reported as 100%.
The INR for healthy people is 1.0 - 1.08.

Control of anticoagulant therapy . The use of INR is especially indicated to determine the therapeutic range in patients using oral anticoagulants. The following table indicates the target results that must be obtained related to several anticoagulant therapeutic indications:

Therapeutic indication

Pre and post-anticoagulation (initiated 2 weeks earlier)	INR	
	Target	Variation
Rib surgery	2.5	2.0 - 3.0
Other surgeries	2.0	1.5 - 2.5
Prevention of primary or secondary venous thrombosis	2.5	2.0 - 3.0
Active venous thrombosis, pulmonary embolism, prevention of recurrent venous	3.0	2.0 - 4.0
Prevention of arterial thromboembolism and patients using mechanical heart pumps	3.5	3.0 - 4.5

INR above 5.0 is strongly associated with an elevated risk for hemorrhage.

According to WHO[1], all results, regardless of test indication, must be released as Activity (A%) and International Normalization Index (INR), and all professionals dealing with oral anticoagulant therapy should be encouraged to use INR, thus abandoning Activity for assessing anticoagulation.

Modification of oral anticoagulants action[8] . The action of oral anticoagulants may be modified by other medicaments and physiological alterations:

Increased Action . Phenylbutazone, indometacin, clofibrate, salicylates, etacrynic acid, nalidixic acid, D-thyroxin, probenicid, sulfonamides and antibiotics, diphenylhydantoin, tobultamide, butazones, monoamine oxidase inhibitors, pheniramine, methylphenidate, disulfiram, norethandrolone, quinine, quinidine, dipirone, acetaminophen, propylthiouracil, glucagon, and hepatotoxic drugs.

Decreased Action . Barbiturics (except thiobarbiturics), meprobamates, griseofulvin, estrogen and oral contraceptives, mineral oil, diuretics, colestiramine, and gastrointestinal mucosa irritants.

Performance characteristics[9]

Method comparison . The following results were obtained by comparing the proposed and a similar methods

	Comparative Method	Labtest Method
Number of samples	40	40
Prothrombine Time (seconds)	12.1 - 41.7	11.7 - 40.3
Estimates of the average (seconds)	24.5	24.0
International normalized ratio	0.91 - 4.01	0.94 - 4.06
Regression equation	Labtest Method = 1.056 x Comparative Method - 0.037	
Correlation Coefficient	0.993	

Using the regression equation, the total systematic error estimated for IRN of 1.0 is 2.0%, and IRN of 2.5 is 4.2%.

Study of accuracy . The following results were obtained in studies of accuracy

Repeatability - within run

	n	Mean (IRN)	SD	CV (%)
Sample 1	20	1.0	0.01	1.31
Sample 2	20	2.3	0.03	1.35

Labtest

Reproducibility - run-to-run

	n	Mean (IRN)	SD	CV (%)
Sample 1	20	1.0	0.02	1.56
Sample 2	20	2.5	0.05	1.96

Total error . The total error (random error + systematic error) estimated for IRN of 1.0 is 5.0%, and IRN of 2.5 is 8.0%. The results show that the method conforms the CLIA's specification for total error (≤15.0%)[6].

Clinical significance . The PT is extended on all cases of congenital or acquired deficiency of factors II, V, VII, and X. The acquires deficiency occurs, mainly, in people who uses oral anticoagulants or antibiotics, has ingestion or absorption disturbers of vitamin K, haemorrhagic disease of the newborn, obstructive jaundice,poor intestinal absorption, liver insufficiency, fibrinolysis and intravascular coagulation.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The water in the laboratory to prepare reagents and use in the measurements must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be <0.1mg/L. When the deionizitation column has its saturated capacity there is a production of alkaline water with liberation of silicates ions and other reducers and oxidative substances that deplete the reagent in a few days or even hours. Therefore a quality control program of the water it is fundamental.

References

1. WHO Expert Committee on Biological Standardization. 48th Report. WHO Tech Rep Ser 1999, 889:70-95.

2. Quick AJ, Leu M. J Biol Chem 1937, 119:73-84.

3. International Committee for Standardization in Hematology. Thromb Haemostas 1976, 36:237-238.

4. CLSI. Transport, and processing of blood specimens for testing plasma based coagulation assays and molecular hemostasis assay: Approved guide line. 5th ed. CLSI document H21-A5, 2008.

5. NCCLS. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard-Fifth Edition. NCCLS document H3-A5, 2003.

6. CLIA Requirements for Analytical Quality. Available at http://www.westgard.com/clia.htm.

7. Loeliger EA, Poller L, Samama E et al. Thromb Haemostas 1985, 54:515-517.

8. Henrykopf F, Rosenfeld LGM, Guerra CCC. Rev Ass Med Bras 1977, 23:100-102.

9. WHO Expert Committee on Biological Standardization. 33rd Report. WHO Tech Rep Sper 1983, 687:81-105.

10. Labtest: data on file.

Reference

Producto	Reference	Contents
PT	504/5-2	R 1 5 X 2 mL
	504/5-4	R 1 5 X 4 mL

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

Labtest Diagnóstica S.A.
CNPJ: 16.516.296 / 0001 - 38
Av. Paulo Ferreira da Costa, 600 - Vista Alegre - CEP 33400-000
Lagoa Santa . Minas Gerais Brasil - **www.labtest.com.br**
Customer Service | e-mail: customerservice@labtest.com.br

Edition: May, 2016
Ref.: 071117

Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

 **Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests	 **Risco biológico** Riesgo biológico Biological risk
 **Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	 **Marca CE** Marcado CE CE Mark
 **Material Calibrador** Material Calibrador Calibrator Material	 **Tóxico** Tóxico Poison
 **Material Calibrador** Material Calibrador Calibrator Material	 **Reagente** Reactivo Reagent
 **Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)	 **Fabricado por** Elaborado por Manufactured by
 **Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	 **Número do lote** Denominación de lote Batch code
 **Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	 **Controle** Control Control
 **Número do catálogo** Número de catálogo Catalog Number	 **Controle negativo** Control negativo Negative control
 **Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes	 **Controle positivo** Control positivo Positive control
 **Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device	 **Controle** Control Control
 **Liofilizado** Liofilizado Lyophilized	 **Corrosivo** Corrosivo Corrosive
 **Período após abertura** Período post-abertura Period after-opening	 **Uso veterinário** Uso veterinario Veterinary use
 **Instalar até** Instalar hasta Install before	

Ref.: 140214

Labtest