PT LYO 5

IVD

CE

PROTHROMBIN TIME according to QUICK (PT)

REF CO1010 7x5 mL

For in Vitro Diagnostic use

METHOD AND PRINCIPLE

PT LYO 5 is a thromboplastin extracted from rabbit brain, lyophilized to be reconstituted with its own diluent provided in the kit, for the determination of prothrombin time according to Quick. It contains the Tissue Factor, lipids and calcium ions and is used for the study of the extrinsic way of coagulation. PT LYO 5 is a Thromboplastin highly sensitive to Vitamin K antagonists, at low levels of extrinsic pathway factors (Factor II, V, VII and X), to hereditary or acquired coagulation disorders, to liver failure.

When the Calcic Thromboplastin PT LYO 5 is added to the patient's plasma, the formation of a fibrin clot is induced in a measurable time either manually or by optical and mechanical instrumentation.

CLINICAL SIGNIFICANCE

Prothrombin Time or Quick Time (also known as PT) and its derived measures (Ratio, INR and Percent Prothrombin Activity) are measurements of the extrinsic and common pathway of coagulation. It is used to determine the tendency to blood coagulation (preoperative screening), to adjust the dosage of anticoagulant therapy with warfarin, or to better determine the severity of a liver disease and to check the status of vitamin K. PT is useful to evaluate five of the twelve coagulation factors (I-Fibrinogen -, II - Prothrombin, V - Proaccelerin, VII - Proconvertine and X - Protrombinase). All these factors are synthesized by the liver and three of these (II, VII and X) are activated by vitamin K-dependent enzymes. Oral anticoagulants, such as Coumadin - Warfarin, are vitamin K antagonists and as such inhibit the activation of the above coagulation factors. By acting in this way, these drugs "fluidify the blood", preventing the formation of clots in the circulatory stream. PT is used in combination with the determination of the Activated Partial Thromboplastin Time (aPTT), which measures the intrinsic coagulation pathway.

REAGENT COMPOSITION

Reagent: Tissue Thromboplastin extracted from rabbit brain.
Solvent: Buffer with calcium ions and stabilizers**.**

REAGENT PREPARATION AND STABILITY

Before to be dissolved, the Reagent, if stored at 2-8 ° C is stable until the date shown on the label.

Reconstitute a bottle of lyophilized Reagent with the entire 5 mL content of a bottle of Solvent. Keep the Reagent so prepared at a temperature of 18-25° C for at least 30 minutes, stirring gently from time to time, avoiding energetic shaking and foaming. The same thing must be done in subsequent uses.

After reconstitution, the Reagent is stable for 8 hours at 37 ° C, 1 day at 22 ° C, 5 days at 16 ° C, 12 days at 2-8 ° C. Do not freeze. For use in multiple analytical sessions of the same bottle, it is recommended to withdraw the necessary volume, to immediately close the bottle and store it in the fridge in order to avoid contamination, degradation from direct light and evaporation. Discard reagent if signs of deterioration appear as failure to recover correct values of certified control plasmas**.**

SPECIMENS

Fresh and decalcified plasma. Take 9 parts of whole blood from a vein and mix with 1 part of trisodium citrate (3.2% - 109 mmol / L). Trisodium Citrate 3.8% - 129 mmol is not recommended. Gently mix, centrifuge (1500 x g for 15 minutes) before performing the test (within 2 hours of sampling) on the supernatant plasma. For further details on withdrawal methods, refer to NCCLS document H3-A3 and H21-A3.

PROCEDURE

For use on automatic systems, refer to the user manual of the instrument.

For use on manual or semi-automatic systems, proceed as follows (it is always advisable to make the determinations in duplicate).

1. Bring a volume of Reagent (Reconstituted Calcic Thromboplastin) sufficient to perform scheduled tests at 37 ° C.
2. Add 100 µL of control plasma, calibration plasma (whole or its dilution) or plasma of the patient to each reaction cuvette.
3. Incubate the reaction cuvettes for at least 2 minutes at 37 ° C.
4. Bring a reaction cuvette into the reading cell, zero the stopwatch, add 200 µL of Reagent (Reconstituted Calcic Thromboplastin) and simultaneously start the stopwatch.
5. Measure the coagulation time

RESULTS

The results can be expressed in 4 ways:

1. **Seconds**, which means the observed clotting time.
2. **Percentage**.

Starting from a Calibration Plasma

MTD PLASMA CAL 14x1 mL - REF CO1120

prepare a series of doubling dilutions with saline solution until 1: 2 - 1: 4 - 1: 8 dilutions are obtained. In case the plasma activity value is certified as 100%, the obtained dilutions of plasmas are with activities of 50%, 25%, 12.5% respectively. For different values of the calibrator, divide the assigned value by 2, by 4 and by 8 respectively. For example, if the calibrator is 96%, the dilutions will be 48%, 24%, 12%. Of each dilution, determine the coagulation time performing the tests at least in duplicate or, better, in triplicate. Construct a Calibration Curve reporting the Times against the Activity percentage (%) and report the times of the unknown samples to obtain the corresponding Activity %. If the instrument used allows it, it is possible to enter the values of the curve for an automatic calculation management. It is also possible to perform further or different dilutions, as required, and to enlarge or modify the values of the curve being set up.

3**. Ratio**, which means the clotting time of the sample divided by the time of normal plasma with activity 100%.

4. **International Normalized Ratio (INR)**, a standardization index recommended by the WHO (World Health Organization). It takes into account the sensitivity of Thromboplastin used, sensitivity expressed with the value of ISI (International Sensitivity Index) which in turn takes into account both the Reagent and the instrument used for reading. The ISI represents the sensitivity of the system in use (instrument + reagent) in relation to the coagulation factors. The lower the value of ISI, the greater the sensitivity the system is. The values of ISI of the Reagent vary from lot to lot and are shown in the sheet attached to the kit. They are different depending on the type of instrumentation.

To calculate the INR, raise the Ratio (base) to the ISI (exponent).

$$\mathbf{INR = Ratio^{ISI}}$$

For example, if the patient has a PT of 36 seconds and if the plasma 100% has a PT of 12 seconds, then:

Ratio = Patient Time / Plasma Time 100% = 36/12 = 3

If the thromboplastin ISI, for the system used, is 1.05:

$$\mathbf{INR = Ratio^{ISI} = 3^{1.05} = 3.17}$$

QUALITY CONTROL

For Internal Quality Control (Precision Verification, Repeatability), use Control Plasmas at the normal (I) and pathological (II) levels:

MTD PLASMA CONT I-II 7 + 7 x 1 mL REF: CO1110

For the evaluation of the Accuracy of the method (Reproducibility) it is necessary to adhere to a program of External Quality Assessment (EQA) managed by certified bodies.

EXPECTED VALUES

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results. The normal values depend on the population of the area in which it operates and on the instrumentation used.

Only as a guideline, they can be considered normal:

• seconds: 11 - 14
• Activity%: 120% - 80%
• INR: up to 1.6

The therapeutic ranges for INR vary from pathology to pathology and can be evaluated by clinicians who follow appropriate therapies.

PERFORMANCES

PRECISION

Precision depends from many factor, such as instrument, reagent and manual skill. Twenty normal samples and twenty abnormal samples were tested and the following CV% were obtained:

Normal Level: Samples= 20; CV = 1,9%

Abnormal Level: Samples= 20; CV = 2,3%

SENSITIVITY

The sensitivity study was carried out by performing scalar dilutions of a plasma considered normal (with activities around 100% and INR around 1). The following results were obtained:

100% = 12.6 "- 50% = 18.5" - 25% = 34.8 "- 12.5% = 43.6"

ACCURACY (CORRELATION)

The comparison between this Thromboplastin (x) and another commercial product (y) gave the following results on 20 different samples:

- Time correlation: r = 0.98 y = 1.16x +1.3
- INR correlation: r = 0.98 y = 0.89x + 0.05

SPECIFICITY / INTERFERENCES

- Sodium oxalate, EDTA and Heparin can not be used as anticoagulants.
- Triglycerides > 700 mg/dL, Bilirubin > 15mg/dL, Hemoglobin > 0.68 g/dL may interefere
- Times may be lengthened if the patient uses oral contraceptives, corticosteroids, EDTA, asparaginase, clofibrate, erythromycin, ethanol, tetracycline and oral anticoagulants such as heparin and warfarin.
- Time may be reduced due to the use of antihistamines, caffeine, phenobarbital and Vitamin K.

NOTE

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

PRECAUTIONS

The products do not contain dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.

However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:

S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.

S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.

S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.

S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.

S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAFY

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