PT CALCIUM THROMBOPLASTIN

Valuation of Prothrombin Time (PT) with Calcium Thromboplastin in plasma use manual and automatic procedure

TEST SUMMARY

Calcium thromboplastin may be used for the determination of prothrombin time (PT) in plasma, either by manual or automatic method that have the characteristics required for the application equipment. Determination PT is sensitive to the deficiency of activities for factors II, V, VII, X, due to hereditary disorders, liver disease, vitamin K and oral anticoagulation.

SAMPLES

Collected, into the plastic or siliconized glass tube, 9 parts of freshly draw venous blood and 1 part of trisodium citrate 3.8%. The plasma was separated after centrifugation of the mixture for 10 'at 1500 x g.
Stability: 2 hours at 15-25°C or 4 hours at 2-8°C.

REAGENTS

Thromboplastin:	calcium thromboplastin from rabbit brain, Polybrene, preservatives.
Buffer:	Hepes buffer, preservatives.

REAGENTS PREPARATION AND STORAGE

Reconstitute one vial of thromboplastin with the contents of a vial of Buffer. Stopper the bottle and mix gently by inversion to avoid foaming. Keep the product at room temperature (15-25°C) for at least 30 '. Before use, mix gently by inversion.
The reconstituted product is stable 7 days at 2-8 ° C, if kept in the original bottle.

MATERIAL REQUIRED BUT NOT SUPPLIED

Test tubes, chronometer, calibrator (where you want to express the results in 'ratio' or activity rate), 'Normal' Control, L Pathological Control, H Pathological Control, Diluent.

PRECAUTION

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general “Good Laboratory Practice” (GLP) guidelines.

PROCEDURE

Manual Application

Place the reconstituted Thromboplastin Reagent incubated at 37 ° C. In test tubes transfer 100 µl of sample and put in thermostat at 37°C for 2 minutes. Add 200 µl of Thromboplastin Reagent after it is mixed gently by inversion.
Value the time clot formation with chronometer started when adding reagent.

INTERPRETATION OF RESULTS

Each laboratory should provide to determining the ISI of the reagent by using a pool of normal plasmas of population as reference. It is still attached to the conversion table of the lot of thromboplastin. Check the INR of the sample according to the seconds obtained from normal calibrator 100%.
When using manual or semiautomatic method, the results in INR are obtained by applying the following formula:

$$INR = (PT\ ratio)^{ISI}$$

EXPECTED VALUES

Every laboratory should be establish own reference intervals in relation to own population.

PT	13-17 sec.
PT (ratio)	0.9-1.2
PT (activity)	120-70%

ISI (International Sensitivity Index)

When the same plasma is analyzed using thromboplastin from different sources or by using different analytical method, you may obtain a significant change in the values of PT.
There is thus a need to provide results of PT uniforms, particularly for patients on oral anticoagulation therapy. The ICSH (International Comittee for Standardization in Hematology) and ICTH (International Committee on Thrombosis and Haemostasis) proposed a method for standardize the results of PT to referring to a peculiarity of each Thromboplastin: sensitivity.
The ISI (International Sensitivity Index) is then used to calculate the INR (International Normalized Ratio).
The value of ISI indicated was determined by calibrating each batch of reagent with a secondary standard, according to the procedure recommended by ICSH / ITCH.
The value of ISI is printed on the vial of thromboplastin

THERAPEUTIC RANGE

International Normalized Ratio (INR)

	Value	Range
a) oral anticoagulant treatment (of at least two weeks) for operation		
Hip	2.0	1.5 - 2.5
Other	2.5	2.0 - 3.0
b) Primary and secondary prevention of venous thrombosis	2.5	2.0 - 3.0
c) active venous thrombosis, pulmonary embolism, prevention of recurrent venous thrombosis	3.0	2.5 - 3.5
d) Prevention of arterial thromboembolism and in patients with mitral valve	3.5	3.0 - 4.0
e) Patients on oral anticoagulant therapy (TAO)		2.1 - 4.8

CLINICAL SIGNIFICANCE

High values are obtain with deficiencies of factors II, V, VII and X (due to congenital anomalies), in states of vitamin K deficiency, liver diseases, in oral anticoagulant therapy, intravascular coagulation, with increased concentrations of antithrombin or antithromboplastin, in fibrinogenolysis in idiopathic myeloid metaplasia

NOTE

- The high sensitivity of the thromboplastin allows accurate monitoring of oral anticoagulation.
- As with any diagnostic procedure, if the results are inconsistent with the clinical presentation, the physician should evaluate data obtained using this test in light of other clinical information.
- Only for in vitro diagnostic use

CALIBRATION / QUALITY CONTROL

Normal and Pathological control are recommended for a complete quality control program.
A pool of normal human plasma can be used as calibrator plasmas as an alternative to commercial plasma. In this case it is important to exclude from the pool of plasmas subjects illness with estrogen-progesterone preparations.

TEST PERFORMANCE

Methods comparison

A comparison with a commercial available product gave the following results in a comparison on 94 samples of serum:

INR Intermedical = y
INR competitor = x
n = 94
y = 0,25648 + 0,77588x r = 0,99532

PT% Intermedical = y
PT% competitor = x
n = 94
y = -6,64531 + 1,00799x r = 0,99262

Precision

INTRA - ASSAY Values in INR			
n = 15	Mean	SD	CV%
Normal INR	1.0206	0.0116	1.14
Middle INR	1.4193	0.0153	1.08
High INR	2.716	0.0188	0.69

INTRA - ASSAY Values in% of prothrombin activity			
n = 15	Mean	SD	CV%
PT% Low	18.9	0.2582	1.36
PT% Middle	47.8	0.9411	1.97
PT% Normal	95.8	2.2103	2.31
INTER - ASSAY Values in INR			
n = 10	Mean	SD	CV%
Pathological INR	1.58	0.02	1.27
Normal INR	1.007	0.0125	1.24

Interferences

Hemolyzed plasma can give difficult results. Were detected interference from drugs. Further tests will be needed to determine the cause of unexpected abnormal results. Concentrations of heparin up to 0.5 U/mL do not interfere.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CG-03

Thromboplastin	4 x 5 ml (liophile)
Buffer	4 x 5 ml (liquid)

REFERENCES

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