THROMBOPLASTIN DS

Prothrombin Time according Quick (PT)

REF. 1001 10x 4 ml
REF. 1001/12 12x 4 ml
REF. 1016 10x10 ml
REF. 1016/12 12x10 ml

CE
IVD

DNV CERTIFIED COMPANY
UNI EN ISO 9001:2008
UN EN ISO 13485:2012

GIESSE DIAGNOSTICS

PRINCIPLE

The PT is used as a screening tool and as a quantitative test for coagulation factors in the extrinsic and common pathways. This test will be prolonged in patients with acquired or congenital disorders that reduce the activity of factors I (fibrinogen), II (Prothrombin), V, VII, and X. The PT is also widely used to monitor oral anticoagulant therapy. Oral anticoagulants reduce the activity of vitamin-K dependent clotting factors (II,VII, IX, X, Protein C, and Protein S) and the PT is prolonged as a result.

The PT measures the clotting time of plasma after adding a source of tissue factor (thromboplastin) and calcium. The recalcification of plasma in the presence of tissue factor generates activated Factor Xa (F.Xa). F.Xa in turn activates Prothrombin, which converts fibrinogen to an insoluble fibrin clot.

SAMPLE

Plasma in trisodium citrate 3.2 % (0.109 M).

Avoid hemolysis and contamination by tissue fluids. Centrifuge blood for 15 minutes at 1500 x g. Test within 2 hours se if samples are held at 22-24°C. If testing is not completed within 24 hours, plasma should be frozen at -20 °C for up to 2 weeks or at -70 °C for up to 6 months.

KIT COMPONENTS

Reagent Lyophilized	Rabbit brain tissue	2 %
	Buffers	5 %
	Sodium azide	0.013 %

The reagent is stable until the expiration date indicated on the label if stored tightly closed at 2-8°C .

REAGENT PREPARATION

Reconstitute the vial of Reagent with 4 ml (Ref. 1001) or 10 ml (Ref. 1016) of distilled water.

Mix gently and let the vial stand undisturbed for 15 minutes at room temperature. After reconstitution, the reagent is stable for 7 days if stored at 2-8°C, for 24 hours at room temperature (15-25°C) and for 8 hours at 37 °C.

Do not freeze

PRECAUTIONS AND WARNINGS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Use the normal precautions required in the laboratory.

Dispose of waste according to local laws.

PROCEDURE

Dispense the sample into a plastic tube or siliconized glass as shown in the scheme:

Sample	100 µl
Incubate for about 2 minutes at 37°C. Add:	
Reagent (prewarmed at 37°C)	200 µl
Time clot formation.	

Test in duplicate (the difference between 2 values must be ≤ ± 5 %, otherwise make a third test).

RESULTS INTERPRETATION

It' s possible to word Prothrombin Time in three ways: **Percentage (Quick Factor); Ratio; I.N.R. (International Normalized Ratio).**

1. PERCENTAGE

Prepare 5 dilutions of a pool of normal plasma as in the table:

Dilution Value %	No one 100 %	1+ 2 50%	1+2 33%	1+3 25%	1+7 12.5%
Plasma	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
NaCl 9 g/l	-	0.5 ml	1.0 ml	1.5 ml	3.5 ml

Of each dilution time clot formation in duplicate or in triplicate with 4 or 5 dilutions (according to the instrument in use).

2. RATIO

$$\text{RATIO} = \frac{\text{Prothrombin Time (Sample)}}{\text{Prothrombin Time (Pool Normal Plasmas)}}$$

3. I.N.R. = RATIO^{ISI}

To standardize testing, the Word Health Organization (WHO) recommends to word the results as INR.

The INR is calculated using the RATIO value according to the following relationship:

I.N.R. = RATIO^{ISI}

For example, with an ISI = 1 and a RATIO = 3.2, INR will be:

INR = (3.2)^{1} = 3.2

The ISI (International Sensitivity Index) is a measure of a thromboplastin / instrument sensitivity to coagulation factors.

ISI values are assigned by comparison to a primary reference material. High sensitivity reagents have low ISI values.

According to WHO recommendations, INR values above 5.5 place the patient at unnecessary risk for bleeding complications. It is generally advised that patients on stabilized oral anticoagulant therapy should be maintained at an INR of 2 - 3.5 depending on the clinical indication.

The ISI value is specific for each Thromboplastin batch.

LIMITATIONS

Technique

The pH will increase if plasma is open to air. Store samples stoppered in plastic or siliconized glass.

Plasma held at 4-8°C may undergo cold activation resulting in significant shortening of the PT.

Thromboplastin DS was designed to work at 37°C ± 0.5°C. Frequently check the temperature of all heating elements.

All labware must be clean and free of trace amounts of detergents.

Interfering Substances

Sodium oxalate, EDTA, and heparin are not suitable anticoagulants.

The PT may be prolonged by substances such as oral contraceptives, corticosteroids, EDTA, asparaginase, erythromycin, ethanol, tetracycline, heparin.

The PT may be shortened by substances including antihistamines, butabarbital, caffeine, oral contraceptives, phenobarbital, and vitamin K.

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EXPECTED VALUES

In multi-center studies, when Thromboplastin DS was evaluated on a normal population, the following results were obtained:

Instrument	PT mean (secs)	Range (+/-2SD)	N
MLA™ Electra 1000C™	13.2	11.4 – 15.0	40
MLA™ Electra 900C™	13.7	12.4 – 15.0	20
IL ACL™ 300/3000+	10.5	8.9 – 12.1	61
Amelung KC 10 ™	12.7	9.3 – 14.2	20
Pacific Hemostasis ThromboScreen 400C	13.5	12.2 – 14.8	38
Pacific Hemostasis ThromboScreen 200	13.5	12.0 – 15.1	60

These values are intended as a guideline only. Each laboratory should establish a Normal Reference Range (NRR) using instrumentation, blood collection methods, and testing techniques used in that laboratory. The NRR should be reestablished or at least verified when changing lot numbers of the same reagent. A new NRR should be established with any change in reagents, instrumentation, blood collection techniques, or anticoagulant.

The clotting time of abnormal plasmas will depend on the ISI of the reagent lot in use.

QUALITY CONTROL

Normal and abnormal plasmas should be tested in conjunction with patient plasmas.

PERFORMANCE

Precision: Precision of Prothrombin Time results is dependent on many factors, such as the instrument, technique and the reagent in use. Thromboplastin DS precision was assessed by testing a normal and abnormal plasma on several different instruments. Asummary of the results follows.

Precision within-run, %CV (N=20)

Sample	MLA Electra 1000C	ThromboScreen 400C	ThromboScreen 200	Amelung KC10
Normal	1.1 %	1.9 %	1.9 %	2.9 %
Anormal	2.8 %	2.5 %	2.3 %	1.1 %

Sensitivity: Thromboplastin DS detects deficiencies in the extrinsic pathway as determined by the Prothrombin Time test. Factor sensitivity testing was performed by diluting pool normal plasma with factor deficient plasmas such that the final factor concentration ranged from10 - 100 %. PT testing of these samples was performed on the MLA-1000C instrument.

	Prothrombin Time (secs)			
% Factor	Factor II	Factor V	Factor VII	Factor X
100	11.6	11.6	11.8	11.7
50	11.6	13.2	12.6	12.8
40	11.7	13.9	12.8	13.3
30	12.3	14.9	13.5	14.1
20	12.8	15.9	13.9	14.8
10	14.1	18.3	15.2	17.0

Correlation: Correlation studies were performed against two other sensitive thromboplastin reagents by performing PT testing on normal and abnormal samples.

	PT Correlation	INR Correlation
Thromboplastin DS vs. Reagent A, (N=49)	R = 0.98 y = 1.16x + 1.30	R = 0.98 y = 0.89x + 0.05
Thromboplastin DS vs. Reagent B, (N=49)	R = 0.95 y = 1.01x + 2.20	R = 0.95 y = 0.82x + 0.10

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