PT-HS CE

CONTENTS			
REF	3510101	PT-HS	10 x 5 mL
For *in vitro* diagnostic use only			

PT-HS

Prothrombin Time (PT)

PT with Calcium

High-Sensitivity Reagent

PRINCIPLE

The capacity of blood to form a fibrin clot by means of the extrinsic hemostatic pathway requires thromboplastin, calcium, factors I, II, V, VII and X [4,5]. The PT-HS reagent provides a source of tissue thromboplastin and calcium that specifically activates factor VII in the extrinsic coagulation pathway. The factors involved in the intrinsic coagulation pathway are bypassed[6].
Therefore, deficiencies of intrinsic pathway factors (VIII, IX and XII) are not detected using the PT test.

REAGENT COMPOSITION

PT-HS **Lyophilized thromboplastin** of rabbit brain and $CaCl_2$. Sodium azide 0.05%.
International Sensitivity Index (ISI): 1.07 – 1.39.

DIL **PT Diluent**

Optative. **Plasma Control Level 1** Ref. 3520101
Plasma Control Level 2 Ref. 3520201.

STORAGE AND STABILITY

Store at 2-8ºC.
The reagents are stable until the expiry date stated on the label. The reconstituted PT-HS reagent is stable for 8 hours at 37ºC or 12 days at 2-8ºC, 1 day at 20-25ºC or 5 days at 15-19ºC stored in the original container. Do not freeze.

REAGENT PREPARATION

1. Reconstitute the contents of **PT-HS** with one vial of **PT Diluent.**
2. Keep the reagent warm at 37ºC for least 30 minutes for proper reconstitution. Swirl the vial gently 5-10 times before use, do not shake. Wait until the reagent reaches the working temperature.
   Using of stirring bar is necessary during the measurement.

SAMPLES

- PT-HS test requires freshly decalcified plasma. To obtain it, mix nine parts of freshly drawn venous blood with one part trisodium citrate (3,2%; 109 mmol/L). The use of higher concentration of trisodium citrate (3,8%;129mmol/L) is not recommended.
- Mix the blood carefully and centrifuge plasma before testing.
- The measurement must be performed within 24 hours. Do not store the sample at 2-8ºC. Refer to Clinical and Laboratory Standards Institute (CLSI) guidelines H21-A5.

INTERFERENCES

- Heparin (<0.75 IU/mL) does not interfere.
- Bilirubin (< 270 mmol/L) does not interfere.
- Hemoglobin (< 6.8 g/L) does not interfere.
- Triglycerides (<9 mmol/L) does not interfere.
- Other drugs and substances may interfere.[10]

ADDITIONAL EQUIPMENT

- Coagulometer or stopwatch and bath at 37ºC ± 0.5ºC.
- General laboratory equipment.

PROCEDURE

This procedure pertains to manual or semi-automated coagulation systems. Refer to your instrument manual for more detailed instrument specific instructions.

1. Pre-incubate the reconstituted PT reagent to 37ºC for at least 10 minutes.
2. Pipette **50 µL** of sample or control plasma into a test
3. cuvette
4. Incubate at 37ºC for 2 minute.
5. Rapidly add **100 µL** of the pre-incubated PT reagent, simultaneously starting the timer.
   Record the clotting time in seconds.

For semi-authomatic system refer to your instrument manual.

CALCULATIONS

Calculate the mean clotting time of duplicate samples and controls. Differences between duplicate results should be less than 5%. Repeat the test if necessary.

The PT result may be reported as 4 different ways:

1. Seconds, observed clotting time.

2. PT ratio (PR):

$$\text{PT ratio (PR)} = \frac{\text{PT of the patient in sec}}{\text{PT of normal plasma in sec}}$$

3. Percent activity. the proportional part of the normal PT activity, which is calculable from the calibration curve. Method dependent master curve in the issued sheet can be used for the calculation.

4. International Normalized Ratio (INR)

The INR is recommended for use with patients undergoing anti-coagulation therapy.

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The International Committee for Standardization in Hematology and the International Committee on Thrombosis and Hemostasis have agreed on recommendations for the reporting of Prothrombin Time results as an International Normalized Ratio (INR). The INR is based on the International Sensitivity Index (ISI) of Thromboplastin reagents[7-9].
PT reagents are assigned with an ISI value by calibration against an International Reference Preparation (IRP 67/40) with an assigned ISI of 1.0. The ISI value for each lot of PT reagent appears on the vial label.

The INR is calculated using the following formula:

INR = (Patient PT / Mean Normal PT) ^{ISI}

ISI = Specific International Sensitivity Index for the Reagent/Instrument system. The ISI is lot specific.

Mean Normal PT = Lot specific mean of the normal range, as determined by each laboratory for the Reagent /Instrument System. It is usually based upon the PT mean plus or minus 2 to 3 standard deviations using 20 or more individuals.

REFERENCE VALUES

PT results are influenced by the method of clot detection and can vary from laboratory to laboratory. In general, PT tests performed with normal plasmas will give clotting times in the range of 13 to 15 seconds on a photo-optical coagulometer, from 11 to 15 seconds if a mechanical coagulometer is used, and between 12 and 15 seconds if the manual method is applied. However, each laboratory should establish a normal range using individuals representative of its patient population. A new normal range should be established with any change of instrumentation, blood collection techniques, or anticoagulant. A Mean Normal PT range should be reestablished or verified when changing lots of the same reagent [(11)].
Therapeutic ranges for monitoring oral anticoagulation therapy will vary from laboratory to laboratory. Therefore, it is essential that each laboratory establish relevant PT ranges for its respective patient population.
Abnormal results obtained with a plasma from a patient not on anticoagulant therapy may indicate a factor deficiency or the presence of an inhibitor. The result may also be due to the effects of certain drugs and medications [(12)]. Additional procedures such as an APTT and mixing studies using factor deficient plasma are usually required.
The normal range expressed in INR is 0,8-1,2. Every laboratory should determine its own MNPT value and reference range.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. They should be used as sample.

REF 3520101 PLASMA CONTROL LEVEL 1

REF 3520201 PLASMA CONTROL LEVEL 2

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

The PT reagent is an *in vitro* diagnostic assay intended for use in performing the one stage prothrombin time (PT) test and assays which are based on a modified prothrombin time.
The prothrombin time is the method of choice for monitoring oral anticoagulation therapy[1] and is a fundamental screening test for acquired or inherited bleeding disorders. During oral anti-coagulation therapy, the activity of vitamin K-dependant clotting factors (II, VII, IX, X, Protein C and Protein S) is reduced and PT time is increased. The test is used for quantitative determination of blood clotting factors in the extrinsic (VII) and common pathways (II, V and X) of coagulation[2,3].

ANALYTICAL PERFORMANCE

- Linearity: INR 0.8 – 5.0.

- Sensitivity range: ISI : 1.07 – 1.39.

- Normal PT time mean: 12.0 – 14.7 sec.

- Analytical sensitivity: 3%.

- Traceability: Reference results of WHO, by Standard Rabbit Plain Thromboplastin (WHO Thrombosis Center Leiden, and Univ. Ontario, Canada).

- **Accuracy**: Results obtained with this reagent did not show significative differences when compared with reference reagents. Details of the comparison experiments are available on request.

- ***Precision:***

Sec.	Within-run		Between-run	
Mean	14,9	20,8	14,9	20,5
CV%	0,739	0,786	1,420	3,648
N	10	10	10	10

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- By calculating with inappropriate data or using the supplied data improperly, erroneous results may occur.
- Due to its ingredients the reagents should be handled with care by observing the precautions recommended for biohazards materia.
- Reagent meeting specimens and other materials should be handled as if capable of transmitting infection and should be disposed of with proper precautions.
- This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

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PT CE

MASTER CURVE

Enter the following values in ARES coagulometer

LOT Nº	35181
EXPIRY DATE	2018-11
%	SEC.
100%	11.7 SEC
50%	18.4 SEC
33%	25.3 SEC
25%	31.8 SEC
MNPT	11.7
ISI	1.11

NOTES

- The efficiency of reconstitution of reagent may be improved by performing it at 37ºC.
- Please, enter the following data into the keyboard (See calibration menu in coagulometer manual) Print, and archive the calibration line. This calibration curve is valid for this lot of PT only.
- The mean normal prothrombin time (MNPT) depends on the population, race, gender, sampling tube etc. Our value, that is identical with the 100% point of the calibration curve is for information only.

  According to the CLSI every laboratory should determine it's own MNPT.

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