CE

IVD

PT-Phen™ LRT

REF CK584K, R 6 vials x 5 mL
REF CK584L, R 12 vials x 5 mL
REF CK586K, R 6 vials x 20 mL
REF CK586L, R 12 vials x 20 mL

HYPHEN BioMed
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English, revision: 04-2021

INTENDED USE:

Clotting method for the *in vitro* quantitative determination of Prothrombin Time (PT), in human citrated plasma, using an automated method. This global method is for the screening of the coagulation disorders (extrinsic pathway) and the monitoring of Vitamin K Antagonists (VKAs) treatments.
This therapy is currently used for curative or preventive indications of thrombotic diseases.
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical:[1,2]
PT is a one-stage test based on the Quick method. PT assesses the overall activity of clotting factors of the extrinsic pathway (coagulation triggered by contact with thromboplastin expressed by specific cells).
PT does not explore factor deficiencies of the intrinsic coagulation pathway (Factors VIII, IX, XI, and XII), platelets, Factor XIII or natural inhibitors of coagulation (Antithrombin, Protein C and Protein S).
Clinical:[2,]
The PT test is used to explore abnormalities of factors II, V, VII, X and fibrinogen (Fbg) in bleeding disorders, liver disease, vitamin K deficiency, disorders of fibrinolysis and disseminated intravascular coagulation (DIC).
PT test is the most commonly used coagulation assay for laboratory monitoring of anticoagulant therapy by VKAs.
Any isolated prolongation of PT, not associated with VKA therapy, has to be explored by individual assay of factors involved in the extrinsic coagulation pathway (FII, FVII, FV, FX).

PRINCIPLE:

PT is the determination of a clotting time at 37°C in the presence of thromboplastin and calcium, by initiation of activation of the extrinsic coagulation pathway (complex TF-FVIIa activated factor X, then generation of thrombin, leading to fibrin formation). The clotting time depends on the activity of extrinsic pathway coagulation factors (II, V, VII, X, Fbg). Measured PT can be converted to concentration (PT%), or expressed as a ratio (PT ratio), or as recommended "International Normalized Ratio (INR)".
The reagent includes an heparin neutralizing substance.

REAGENTS:

R **PT-Phen LRT reagent,** at approximately 200 ng/mL of recombinant human Tissue Factor (rec(h)TF), liquid form. Contains BSA, a specific heparin neutralizing substance, preservatives and stabilizers.

The ISI for the reagent lot used (near 1) is indicated on the flyer provided with the kit.

The product is classified as non-hazardous and is not subject to labeling according to EC Regulation No. 1272/2008 [CLP]

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.

REAGENT PREPARATION:

R Reagent is ready to use; homogenize while avoiding formation of foam and load it directly on the analyzer following Application Guide instruction.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **1 month at 2-8°C.**
- **Stability on board of the analyzer: see the specific Application Guide.**

Combination of storage are not recommended.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Imidazole Buffer (AR021B/AR021K/AR021L/AR021M/AR021N) (optional).
- Specific calibrators and controls :

Product Name	Reference
EASYPLASMA™ Control Set	225601
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301
PT Calibrator Set	SC084K
Reference Plasma Pool (optional)	NA

- Automatic analyzer for clotting assays such as: CS-series, CN-series.
- Laboratory material.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose is not modified.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.
Samples should be collected, prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[5] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references [5,6].

PROCEDURE:

HYPHEN BioMed provides Application Guides for defined coagulation analyzer families. The Application Guides contain analyzer/assay specific handling and performance information and supersede the information in these Instructions for Use.
Enter ISI value, and MNPT as needed, for each new lot.

TRACEABILITY:

The International Sensitivity Index (ISI) value assignment of each PT-Phen™ LRT lot is determined against an in house standard, related to the corresponding WHO International Standard and using patients under VKA therapy[2]. Certificate of traceability is available upon request.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- The prothrombin time obtained (PT seconds) may be converted to percent of normal value (PT%), expressed as a ratio (PT ratio), or as "International Normalized Ratio (INR)".
- It is recommended (WHO organization) to report the PT values in INR, especially for patients treated with VKA.
- The principles are as follows [2,3,4].

Direct determination of patient INR or PT% value [3,4]
Realize an INR (or PT%) reference calibration curve by determining the clotting times of PT Calibrator Set (SC084K) and plotting log-log on abscissae the INR (or PT%) values and on ordinates the corresponding clotting times (seconds). The INR (or PT%) value of tested plasma is directly obtained on the calibration curve.
This method is independent of International Sensitivity Index (ISI) and MNPT values.

INR equation
The INR value can be determined by calculation method. This method depends on the ISI and MNPT values using following equation[2,4]:

$$INR = \left[\frac{PT(sec)\ plasma}{MNPT} \right]^{ISI}$$

With MNPT = Mean Normal Prothrombin Time (geometric mean from at least 20 local healthy adult normal plasmas, or local citrated human reference plasma pool, clearly established and validated as Normal Control, as per current local recommendations or guidelines [2])
And ISI = International Sensitivity Index, for the combination of reagent lot/ method or instrument used, determined by reference to WHO reference recombinant human thromboplastin.

Prothrombin time ratio
PT Ratio = (PT(sec)plasma) / (MNPT)

- Lot to lot variability measured on 3 lots is: %CV < 7%.
- The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting no limpid appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- User defined modifications are not supported by HYPHEN BioMed as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in HYPHEN BioMed Application Guides or these Instructions for Use.
- Mechanical or photo-optical systems can be used with PT-Phen™ LRT reagent, however the clot detection method may vary from one to another[4], so it is recommended to not compare the results obtained with different detection methods. Also, results obtained with different thromboplastins, especially from different origin, may vary [4]. According to the method and the instrument used in the laboratory, a local ISI determination may be required[4].
- Many drugs, anti-thrombin and anti-Xa inhibitors, and common variables may affect PT results [2,4]: any unexpected abnormal result should be further explored. The reagent includes an heparin neutralizing substance and is insensitive to heparins up to 1 IU/mL (UFH/LMWH).

EXPECTED VALUES:

VKAs are absent from normal plasma. Normal range, therapeutic range and risk range (according to the drug) should be defined according to the current local recommendations and for the specific indication.
Many variables may affect clotting times (eg working conditions such as temperature, water quality, pH, system used, sampling, storage, population)[2]
A normal range study was performed on each analyzer and is documented in the respective Application Guides of the analyzers.
However, each laboratory has to determine its own normal range in its specific test conditions.

PERFORMANCES:

Performances studies were conducted as described in CLSI guidelines.
The following performance data represent typical results and are not to be regarded as specifications for PT-Phen™ LRT.
Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.

Analytical performances
Measuring Range
The measuring range is defined by the analyzer system used and is documented in the respective Application Guides of the analyzers.
Precision
Precision studies were assessed using laboratory controls and spiked pooled plasmas over a 20-days period, 2 series per day and 2 repetitions within each series for a sample level. Coefficient of variation (CV) for all samples is less than 3% and is documented in the respective Application Guides of the instruments.
Interfering substances
No significant interference is expected for heparins up to 1 IU/mL (UFH/LMWH), as the reagent contains an heparin neutralizing substance.
Interferences are defined by the analyzer system used and are documented in the respective Application Guides of the analyzers.

Clinical performances

Agreement

	CS-series		
Analyte	**Linear regression**	**r**	**Reference/comparison method**
PT sec	y = 1.30 x – 2.21	0.994	Dade® Innovin
PT %	y = 0.83 x + 8.44	0.988	Dade® Innovin
PT INR	y = 1.01x + 0.05	0.993	Dade® Innovin

Clinical performance was defined on normal plasmas and plasmas from patient with VKAs treatment.

REFERENCES:

1. Quick AJ and al. The nature of the bleeding in jaundice, *JAMA* 1938.
2. Clinical and Laboratory Standards Institute (CLSI). One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline - Second Edition CLSI document H47-A2, Vol. 28 No. 20. 2008.
3. Poller L. *et al.* The prothrombin time/international normalized ratio (PT/INR) Line: derivation of local INR with commercial thromboplastins and coagulometers – two independent studies. J. Thromb Haemost. 2011.
4. Clinical and Laboratory Standards Institute; Procedures for validation of INR and Local Calibration of PT/INR Systems; H54-A, Vol.25, number 23.
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
6. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.

REF	Catalog number	LOT	Batch code	IVD	*In-vitro* diagnostic medical device
Rx	Numerical < x> identification of reagent		See instructions for use	CE	CE marking of conformity
	Temperature limitation		Manufacturer	YYYY-MM-DD	Use by
CONTENTS	Contents	→	Reconstitution volume	EXP	Expiration date
CONTAINS	Contains	i-MA	See instructions in Method Application guide		

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