CE
IVD

(h)PT-Phen (18)

Ref CK583 (12 x 18 mL)

Calcium Thromboplastin for the determination of prothrombin time (PT)

HYPHEN BioMed

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INTENDED USE:

(h)PT-Phen (18) reagent is a thromboplastin reagent (containing recombinant human tissue factor, synthetic phospholipids and calcium at an optimized concentration) for the determination of prothrombin time (PT), in human citrated plasma, using a clotting method, manual or automated.

SUMMARY AND EXPLANATION:

The prothrombin time (PT) is a one-stage test based on the Quick method. The PT assesses the overall activity of clotting factors of the extrinsic pathway (coagulation triggered by contact with thromboplastin expressed by specific cells). This test is used to explore abnormalities of factors II, V, VII, X and fibrinogen in congenital or acquired bleeding disorders), liver disease, vitamin K deficiency, disorders of fibrinolysis and disseminated intravascular coagulation. PT test is the most commonly used coagulation assay for laboratory monitoring of anticoagulant therapy by vitamin K antagonists (VKA).Any isolated prolongation of PT, not associated with Vitamin K Antagonist (VKA) therapy has to be explored by individual assay of factors involved in the extrinsic coagulation pathway (FII, VII, V, X).
PT does not explore factor deficiencies of the intrinsic coagulation pathway (Factors VIII, IX, XI, and XII), platelets, Factor XIII or natural inh bitors of coagulation (Antithrombin, Protein C and Protein S).

ASSAY PRINCIPLE:

Prothrombin time is the determination of a clotting time at 37°C in the presence of thromboplastin and calcium. Complex tissue factor-FVIIa activated factor X, then generation of thrombin, leading to fibrin formation. The clotting time is depending on the activity of extrinsic pathway coagulation factors. The measured PT can be converted to concentration or expressed as "International Normalized Ratio (INR)".

REAGENTS:

(h)PT-Phen reagent:
Reagent, containing recombinant human tissue factor, synthetic phospholipids, calcium ions and a heparin neutralizing agent, lyophilized in presence of stabilizers - 12 vials of 18 mL.
The ISI for the reagent lot used is indicated on the flyer provided with the kit.

CAUTIONS AND WARNING:

- Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.
- The disposal of waste materials must be carried out according to current local regulations.
- Use only reagents from kits with the same lot number.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange. Evaporation reduces reagent stability on instrument board.
- Reagent vials are lyophilized and therefore closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- For in vitro diagnostic use.

PREPARATION, STABILITY OF REAGENTS AND STORAGE:

PT-Phen reagent:
Reconstitute each vial with exactly 18 mL of distilled water. Shake thoroughly until complete dissolution of the content. Let homogenize for 30 minutes at room temperature (18-25 °C), while shaking the vial from time to time.
Homogenize the content before each use.
Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial or in a closed plastic microcentrifuge tube:

- **4 weeks** at 2-8°C.
- **7 days** at room temperature (18-25 °C).
- **Do not freeze.**

STORAGE CONDITIONS:

Reagents must be stored at 2-8°C, in their original packaging box. They are then usable until the expiration date printed on the box

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferentially sterile.
- Imidazole buffer (#AR021A) if a calibration curve is required.
- Specific calibrators and controls (if a calibration curve is required):

Product Name	Reference
EASYPLASMA™ Control Set	225601
Reference Plasma Pool	NA

Also refer to the specific application guide of the analyzer used.

Materials:

- Semi-automatic or automatic coagulation instrument or electromagnetic water bath.
- Stop watch.
- Calibrated pipettes.

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations (In the USA, refer to NCCLS/CLSI document for further instructions on specimen collection, handling and storage).

- Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood.

- Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M) in order to avoid any activation, through a net venipuncture. The first tube must be discarded.

- Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

- Storage of plasma:
  - 4 hours at room temperature (18-25°C)
  - 1 month at -20°C.
  - 18 months at -70°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

TEST PROCEDURE:

Automated methods:

Applications to the various analyzers are available upon request. **Refer to each specific applications and specific cautions for each instrument.**

Manual method:

Principle: a mechanical coagulation indicator, such as a stir bar is used for detecting clotting. The test is performed at 37°C.

Prewarm a sufficient volume of reconstituted (h)PT-Phen at 37°C (0.2 mL needed per test). Take cautions to prevent evaporation and do not hold at 37°C uncapped for longer than 60 minutes. Discard reagent that has been held at 37°C for 60 minutes or longer.

Into a haemolysis test tube or a cuvette, incubated at 37°C, introduce:

- **100 µL** of citrated test plasma

Incubate for 1-2 minutes at 37°C, (introduce the mechanical coagulation indicator if required) and then introduce (Starting the stop-watch):

- **200 µL** of (h)PT-Phen reagent (pre incubated at 37°C).

Record the exact clotting time, in seconds (stop of the stir bar)

If higher or lower reactive volumes than those indicated here above are required for the method used, the same respective proportions between reagent concentrations and sample volumes used, must be adhered to, in order to maintain the assay performances. It is responsibility of the user to validate any modifications and their impact on all assay results.

CALIBRATION:

The calibration line can be realized by diluting a reference citrated normal plasma calibrated and qualified for the test.

The calibration curve (Thivolle line) is prepared by dilution of a reference normal plasma in Imidazole Buffer according to the scheme below.

Percentage	100%	50%	25%	*12.5%
Dilution	Undiluted	1:2	1:4	1:8
Plasma	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Imidazole buffer	0.0 mL	0.5 mL	1.5 mL	3.5 mL

*not recommended on CS instrument.

Refer to the specific package insert of the commercial plasma calibrator.

Draw the Thivolle calibration curve on linear graph paper by plotting on the X-axis the inverse of the dilution (1, 2, 4, and 8) of each cal bration point and on the Y-axis the corresponding clotting time (in seconds). From this cal bration curve, calculate the prothrombin concentration of tested samples (see section Results).

The correspondence table of INR function of ISI for the lot of reagent used is included in the box for each lot on the flyer.

QUALITY CONTROL:

Using quality control plasmas, allows validating the cal bration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents.

Quality control plasmas must be included in each series, as per good laboratory practice, in order to validate generated results. A new calibration curve must be carried out preferentially for each test series, and at least for each new lot of reagents, or after each important analyzer's maintenance, or when quality controls values are measured outside the acceptance range determined for the method.

Each laboratory should establish and verify its own target values, acceptance ranges and expected performances, according to the instruments and protocols used.

RESULTS:

The prothrombin time obtained may be converted to percent of normal value, expressed as a ratio (patient/normal plasma pool), or as International Normalized Ratio (INR).

Results expression in INR is recommended for patients treated with VKA, and should be avoided in the preoperative or hepatic exploration.

Prothrombin time ratio defined as follows:

PT Ratio = (Patient PT) / (MNPT)

Normalized ratio or INR:

INR = ((Patient PT) / (MNPT))^{ISI}

MNPT = Mean Normal Prothrombin Time (local citrated plasma pool, clearly established and validated as Normal Control)

ISI = International Sensitivity Index, determined by reference to WHO reference recombinant human thromboplastin.

ISI value, indicated on the flyer, is specific for the association reagent/instrument. According to the method and the instrument used in the laboratory, a local ISI determination can be required.

The result (in seconds) is converted to a percentage of activity from the calibration curve, or to INR values.

For each batch of (h)PT-Phen, and for each instrument or method used, an ISI value will be determined by recommended reference method (e.g. WHO).

LIMITATION:

- Manual or automatic methods can be used with (h)PT-Phen reagent, however the clot detection method may vary from one to another, so it is recommended to not compare the results obtained with different detection methods.
- No significant interference is observed for bilirubin C concentrations < 25 mg/dL, bilirubin F concentrations < 25 mg/dL, haemoglobin concentrations < 500 mg/dL and triglycerides concentrations < 250 mg/dL added to plasma on CS5100. High levels of haemoglobin or of triglycerides may affect the results.
- The ISI value is specific to each lot and each method or instrument application used (ISI value is about 1). A local ISI determination should be required using certified INR plasma preparations. Each laboratory must define and validate the INR dynamic range for the reagent and the method used, and includes controls at the lower and upper end of this range. A PT/INR calibration range, using INR certified plasmas, is also possible.

EXPECTED VALUE:

The clotting time may vary depending on working conditions such as temperature, water quality, pH, system used, sampling, storage of samples and reagents, population. Each laboratory should establish its own normal range.

PERFORMANCE CHARACTERISTICS:

- There is no sensitivity to Heparin (UFH/LMWH) until 1.5 IU/mL.
- Example of reproducibility data obtained with normal plasma, using the CS5100 instrument:

Control	Within-Run			Between-run		
	n	CT CV %	PT % CV %	n	CT CV %	PT % CV %
Normal	30	0.6	1.1	10	0.9	1.6
Abnormal	30	0.8	1.0	10	0.7	1.1

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SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1.

Changes compared to the previous version.

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