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HEMOCLOT VII-X

 # CK051K / CK051L

Measurement of Factor VII + X with a clotting method

For in vitro use only

Last revision : 11/02/2015

1. Intended use:

The **HEMOCLOT VII-X** kit is proposed for the measurement of Factors VII+X activity in human citrated plasma using a clotting method, triggered with calcium thromboplastin.

2. Assay principle:

The **HEMOCLOT VII-X** method is a clotting assay where all the extrinsic pathway clotting factors are present and in excess, excepted for factors VII and X, which are brought by the diluted tested plasma, and thromboplastin.

Factors VII and X are the limiting factors and clotting time is inversely proportional to the concentration of factors VII and X. There is an inverse linear relationship, on a bilogarythmic graph paper, between the factor VII-X concentration and the corresponding clotting time.

3. Assay specimen:

Human plasma obtained from Trisodium Citrate anticoagulated blood.

4. Reagents:

Each kit contains:

- 6 vials (**ref CK051K**) or 20 vials (**ref CK051L**) of 1 ml of **HEMOCLOT VII-X** reagent, a clotting mixture containing highly purified bovine prothrombin, fibrinogen and Factor V, lyophilized in presence of preservatives and stabilizers.

5. Reagents and material required, but not supplied:

- Pipettes with dispensing volumes of 20 µl, 50 µl and 100 µl.
- Pipette with a variable dispensing volume from 50 µl to 1,000 µl.
- Semi-automatic or automatic coagulation instrument, or fibrometer or electromagnetic water bath.
- Distilled water.
- Imidazole buffer (# AR021A/AR021K/AR021L).
- Normal pool human plasma or Factors VII + X calibrator (BIOPHEN Plasma Calibrator - # 222101).
- Normal and Abnormal control plasmas, titrated for factors VII+X, (BIOPHEN Normal Control Plasma - #223201 and BIOPHEN Abnormal Control Plasma - #223301).
- Calcium Thromboplastin (such as rabbit brain thromboplastin).

6. Reagent preparation and stability:

In their original package, and before any use, when stored at 2-8°C, the **HEMOCLOT VII-X** reagent is stable until the expiration date printed on the kit.

- **Reagent Preparation**:

Hemoclot VII-X: Restore the Hemoclot VII-X vial with 1 ml of distilled water; let for 15 min. at room temperature; mix gently until complete dissolution of the content.

- **Reagent stability following reconstitution:**

– 24 hours at room temperature (18-25°C).
– 72 hours at 2-8°C.
– 1 month frozen at -20° or below.

Nota: Source bovine plasma used for the extraction of proteins included in the preparation of **HEMOCLOT VII-X** were tested with registered methods and found negative for bovine infectious diseases, notably for the bovine spongiform encephalopathy. However, no assay may warrant the total absence of infectious agents. Any product of bovine origin must then be handled with all the required cautions, as being potentially infectious.

7. Sample collection and preparation:

Blood (9 vol.) must be collected on 0.109M trisodium citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 15 min. centrifugation at 2,500 g; citrated plasma must be tested within 8 hours when stored at room temperature (18-25°C), or can be used within 24 hours if kept at 2-8°C, or it can be frozen at –20°C or below for up to 6 months. Just before use, the plasma must be thawed for 15 min. in a water bath at 37°C. Thawed plasma must be used within 4 hours, at room temperature (18-25°C).

8. Protocol:

- **Calibration curve:**

Prepare 2 ml of normal citrated human pooled plasma **diluted 1:10** in Imidazole buffer. By definition, this ten fold dilution of the normal citrated human plasma pool corresponds to a concentration of **100% of factors VII + X.** Using this preparation, the calibration curve is obtained as follows:

VII-X	0%	12.5%	25%	50%	100%
Dilution	**0**	**1:80**	**1:40**	**1:20**	**1:10**
Plasma pool 1:10	0 mL	0.125 mL	0.250 mL	0.500 mL	1 mL
Imidazole Buffer	1 mL	0.875 mL	0.750 mL	0.500 mL	0 mL

The calibration curve must be used within 4 hours at room temperature.

The calibration curve can also be established with the BIOPHEN Plasma Calibrator (#222101), using the factor VII-X activity indicated on the flyer for the lot used.

- **Preparation of tested plasma:**

Tested plasma must be **diluted 1:10** in Imidazole buffer. The diluted plasma must be tested within 4 hours.

- **Assay:**

Manual Method:

Preincubate Calcium Thromboplastin at 37°C.

In a test tube, or a cuvette, introduce:

- 100 µl of **HEMOCLOT VII-X** reagent.
- 100 µL of calibration solution or of tested plasma diluted **1:10**.

Incubate for 1 to 2 min. at 37°C, and then introduce:

- 150 µl of Calcium Thromboplastin preincubated at 37°C.

Record the clotting time.

Automatic Method:

The assay can be used with the semi-automatic or automatic instruments, such as ACL, STA, STA-R, KC-4, KC-10, BCT, BCS, etc…

The usual program used for testing the factors involved in the extrinsic pathway with a clotting based calcium thromboplastin method, and a specific deficient plasma, can be applied. The respective specimen and reagent volume ratios indicated for the manual method must be strictly respected. Usually, with automatic methods the volumes used for reagents and tested plasma (diluted 1:10) are half those recommended for the normal method.

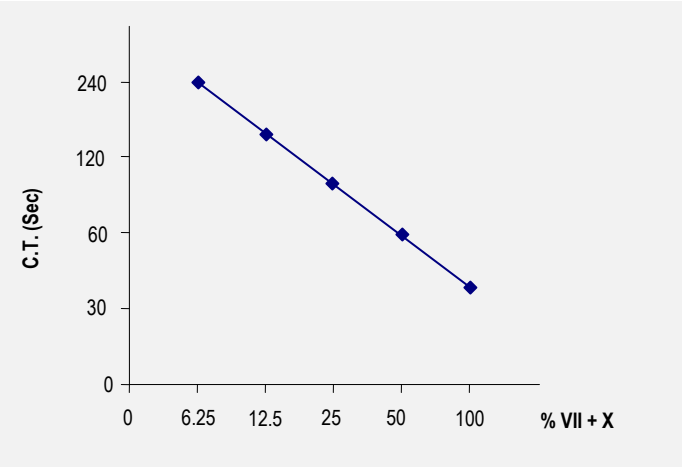
With semi automatic or automatic instruments, especially those with a photometric detection of clot formation, clotting times use to be slightly shorter than with the manual method.

9. Expression of results:

On a bilogarythmic graph paper, plot on abscissae the VII + X concentrations and on ordinates the corresponding clotting times. On the calibration curve obtained, interpolate directly the corresponding VII+X concentration for the tested plasma.

Example of Calibration curve:

This calibration curve is indicated as an example only. It was obtained with Calcium Thromboplastin (Calcic Thromboplastin) from Diagnostica Stago (Neoplastin Cl Plus), using a STAR method.



10. Interferences:

The **HEMOCLOT VII-X** reagents **do not contain heparin inhibitors**. Presence of heparin or of other anti-thrombin or anti-Xa substances may interfere in the assay and prolong the clotting time.

However, current Calcium Thromboplastin preparations use to contain an heparin inhibitor. The assay is then insensitive to the presence of heparin. If any risk of interference of heparin must be avoided, check that the Calcium Thromboplastin used contains an heparin inhibitor. However, heparin inhibitors, present in thromboplastin prepartions, are not always totally efficient for neutralizing Low Molecular Weight Heparin (LMWH).

11. Normal values:

Normal values for factors VII + X activity are usually > 70%.

12. Applications:

The **Hemoclot VII-X** reagent is proposed for measuring factors VII+X, altogether, in any clinical situation where one or both of these factors can be deficient.

The major applications are:

- Vitamin K deficiency (hepatic diseases, primary biliary cirrhosis, deficiency in new-borns, antibiotherapy, …)
- Vitamin K antagonists (dicoumarol therapy, …)
- Isolated deficiencies of factors VII or X.
- Accelerated clotting factor consumption (DIC)

13. Assay variations:

The clotting times observed for this assay are obtained with Calcium Thromboplastin from Diagnostica Stago (Neoplastin Cl Plus). They are in the range from 35 to 45 seconds for the 100% VII+X concentration. The assay performances can slightly very according to the thromboplastin reagent type and lot, and the instrument used in the laboratory. Performances, as well as target values and acceptance ranges for each new lot of quality controls used, must then be confirmed (and adjusted if necessary) in the laboratory working conditions.

14. References:

1. Soulier JP, Larrieu MJ. Etude analytique des temps de Quick allongés. Dosage de prothrombine, de proconvertine et de proaccélérine. Sang 1952 ; 23: 549-559.
2. Favre-Gilly J, Belleville J, Croizat P, Revel L. Les états hémorragiques acquis par trouble plasmatique de coagulation. Cah Med Lyonnais 1967 ; 43 (28) : 2611-2668.
3. Gjonnaess H, Fagerhol MK. Studies on coagulation and fibrinolysis in pregnancy. Acta Obste Gynecol Scand 1975; 54: 363-367.
4. Andrew M, Paes B, Milner R, Hohnston M, Mithell L, Tollefsen DM, Powers P. Development of the human coagulation system in the full-term infant. Blood 1987; 70: 165-172.