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HEMOCLOT Factor V Reagent

CK071K-RUO

Measurement of Factor V activity with a clotting method

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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1. Intended use:

The **HEMOCLOT FV reagent** kit is proposed for the measurement of Factor V activity in human citrated plasma using a clotting method, triggered with calcium thromboplastin. **This kit is for research use only and should not be used for patient diagnosis or treatment.**

2. Assay principle:

The **HEMOCLOT FV reagent** method is a clotting assay where all the extrinsic pathway clotting factors are present and in excess, excepted Factor V, which is brought by the diluted tested plasma, and thromboplastin.

Factor V is the limiting factor and clotting time is inversely proportional to the concentration of factor V. There is an inverse linear relationship, on a semi-logarithmic graph paper, between the factor V concentration and the corresponding clotting time.

3. Assay specimen:

Human plasma obtained from Trisodium Citrate anticoagulated blood.

4. Reagents:

Each kit contains:

- 6 vials of 1 ml of **HEMOCLOT FV reagent**, a clotting mixture containing highly purified fibrinogen and a prothrombin complex concentrate, 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 Factor V calibrator (BIOPHEN Plasma Calibrator - # 222101).
- Normal and Abnormal control plasmas, titrated for factor V, (BIOPHEN Normal Control Plasma - #223201 and BIOPHEN Abnormal Control Plasma - #223301).
- Calcium Thromboplastin (such as rabbit brain thromboplastin).

6. Reagent preparation and stability:

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

- **Reagent Preparation**:

Hemoclot FV reagent: Restore the vial with 1 ml of distilled water; mix gently until complete dissolution of the content (vortex), let for 15 min. at room temperature; homogenize before each use.

- **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.

Note: Source human and bovine plasma used for the preparation of **HEMOCLOT FV reagent** were tested with registered methods and found negative for infectious diseases, especially for BSE (bovine plasma), and for Hepatitis B Surface Antigen, Hepatitis C Antibodies (HVC) and antibodies to HIV 1 and 2 (human plasma). However, no assay may warrant the total absence of infectious agents. Any product of biological 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 (preferentially at 2-8°C); citrated plasma must be tested within 4 hours when stored at room temperature (18-25°C), or can be used within 8 hours if kept at 2-8°C, or it can be frozen at –20°C or below for up to 1 month. Just before use, the plasma must be thawed for 15 min. in a water bath at 37°C.

Refer to GEHT or NCCLS guidelines for further instructions on specimen collection, handling and storage. Discard any plasma presenting an unusual aspect.

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 factor V.** Using this preparation, the calibration curve is obtained as follows:

V	6.25%*	12.5%	25%	50%	100%
Dilution	**1:160**	**1:80**	**1:40**	**1:20**	**1:10**
Plasma pool 1:10	0.060 mL	0.125 mL	0.250 mL	0.500 mL	1 mL
Imidazole Buffer	0.900 mL	0.875 mL	0.750 mL	0.500 mL	0mL

*For a better accuracy in the low range ≤10%.

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

The calibration curve must be used within 2 hours at room temperature.

- **Preparation of tested plasma:**

Tested plasma must be **diluted 1:10** in Imidazole buffer. The diluted plasma must be tested within 2 hours.

Caution : to ensure optimal performances of the assay, perform all assays (calibration, samples, controls) extemporaneously and successively without interruption.

- **Assay:**

Manual Method:

Preincubate Calcium Thromboplastin at 37°C.

In a test tube, or a cuvette, introduce:

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

Incubate for 1 min. at 37°C, and then introduce (starting the stop-watch):

- 200 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 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.

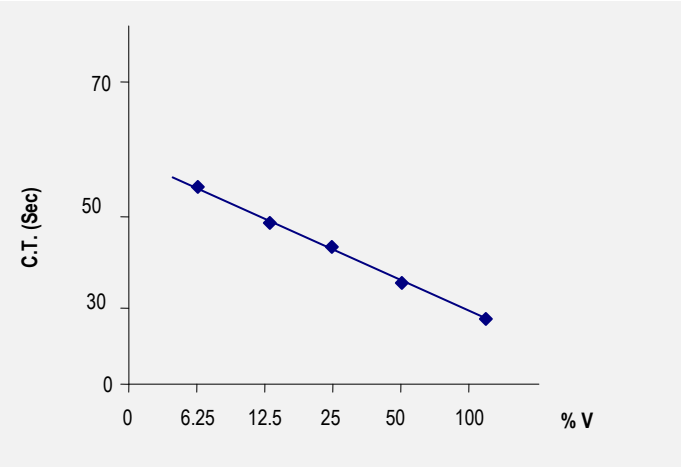
9. Expression of results:

On a semi-logarithmic graph paper, plot on abscissae the FV concentrations (in %) (logarithmic scale) and on ordinates the corresponding clotting times (in sec) (linear scale). On the calibration curve obtained, interpolate directly the corresponding FV concentration for the tested plasma.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

Example of Calibration curve:

This calibration curve is indicated as an example only. It was obtained with Diagnostica Stago Thromboplastin (Neoplastin), using a manual method.



10. Interferences:

The **HEMOCLOT FV** reagent **does 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 preparations, are not always totally efficient for neutralizing Low Molecular Weight Heparin (LMWH).

11. Indicative performances and Assay variations:

The clotting times observed for this assay are obtained with Calcium Thromboplastin from Biomérieux (Calcic Thromboplastin) or from Diagnostica Stago (Neoplastin). They are in the range from 25 to 35 seconds for the 100% V concentration.

Indicative performances obtained in these conditions:
Dynamic range: 6.25-100%
Intra assay CV <4% ; Inter assay CV <7%
Detection threshold : < 10%

For a better accuracy, samples measured ≤10% can be tested at the 1:5 dilution, and obtained results divided by 2; for samples measured >100% (or C%), the 1:20 dilution can be used and obtained results multiplied by 2.

Variable clotting times can be obtained according to the thromboplastins used. The assay performances can slightly vary according to the thromboplastin reagent type and lot, and the instrument used in the laboratory. Performances, as well as the normal range, and 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.