

CE HEMOCLOT Factor V-L Ref: CK061K & CK061L

Measurement of Factor V-Leiden by testing its resistance to the action of Activated Protein C.

For in vitro use only

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INTENDED USE:

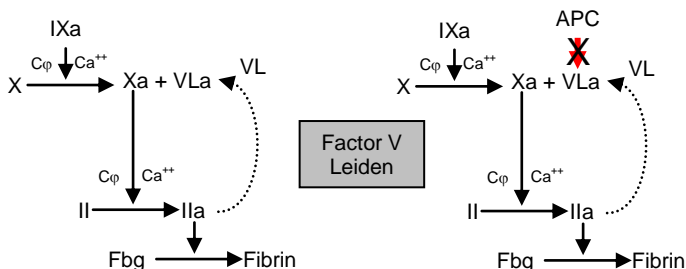
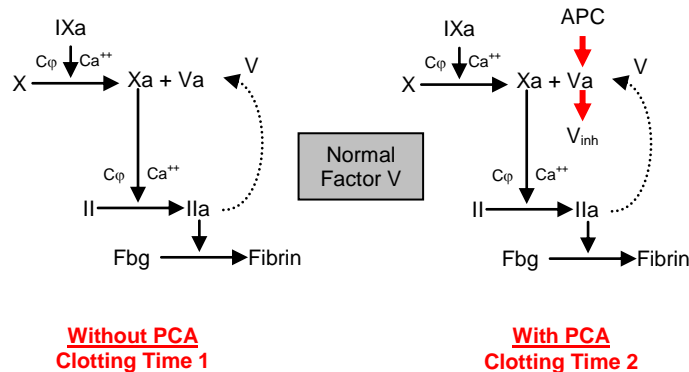
HEMOCLOT Factor V-L Kit is a clotting method proposed for testing the presence of Factor V-L (Factor V Leiden) in citrated plasma, by its resistance to the action of Activated Protein C (APC). The assay is performed in the absence or presence of Activated Protein C. In presence of APC, the prolongation of clotting time is directly related to the concentration of Normal Factor V, and inversely related to the amount of Factor V-Leiden (mutation R506Q).

CLINICAL BACKGROUND:

The Factor V-Leiden is insensitive to the action of Activated Protein C. Its presence induces a prolonged coagulant activity in blood (due to a prolonged survival of Factor Va activity), following activation of coagulation. Patients with Factor V-L (mutation R506Q), are exposed to an increased thrombotic risk. This risk, already present in heterozygous patients, where the Normal Factor V and Factor V-Leiden are both present, is strongly enhanced in homozygous patients, who only have Factor V-Leiden.

PRINCIPLE:

The HEMOCLOT Factor V-L Kit is a clotting method, triggered by purified Factor IXa with Phospholipids and Calcium, performed on the diluted tested plasma, in presence or absence of Activated Protein C. In the first step, the diluted plasma is mixed with purified clotting Factors (Prothrombin and Fibrinogen), in a constant and optimised concentration. Then, the purified Factor X, also in a constant and optimised concentration, is added, without (Clotting Time 1) or with (Clotting Time 2) Activated Protein C (APC). Clotting is initiated by the addition of Factor IXa, in presence of Phospholipids (PLP) and Calcium (Ca^{2+}). Clotting times are then recorded. The ratio of Clotting Times without or with APC (Clotting Time 2 / Clotting Time 1) is calculated. If the plasma is normal, this ratio is ≥ 2.00 , if the plasma is from a patient with the R506Q mutation (Factor V Leiden), this ratio is lowered and ≤ 1.80 .



REAGENTS SUPPLIED: HEMOCLOT Factor V-L (reference CK061K and CK061L) contains the following reagents:

	CK061L	CK061K
R1 : Reagent 1	2 vials of 4 ml	4 vials of 4 ml
R2 A: Reagent 2A	2 vials of 1 ml	4 vials of 1 ml
R2 B: Reagent 2B	2 vials of 1 ml	4 vials of 1 ml
R3: Reagent 3	2 vials of 4 ml	4 vials of 4 ml
Tests to be performed	2 series of 20 patients with the manual method or 2 series of 40 patients with automatic methods	4 series of 20 patients with the manual method or 4 series of 40 patients with automatic methods

R1 : Reagent 1:

Clotting mixture containing human Fibrinogen, human Prothrombin, recombinant Factor VIII:C and Protein S at a constant concentration, optimised for the assay, lyophilised. It also contains an heparin neutralizing substance. Reconstitute each vial with exactly 4 ml of distilled water.

R2 A: Reagent 2A :

Purified Human Factor X, at a constant concentration, optimised for the assay, lyophilised in the presence of rabbit brain phospholipids (cephalin). Reconstitute each vial with exactly 1 ml of distilled water.

R2 B: Reagent 2B :

Purified Human Factor X, at a constant concentration, optimised for the assay, (the same than R2A), containing human Activated Protein C, lyophilised in the presence of rabbit brain phospholipids (cephalin). Reconstitute each vial with exactly 1 ml of distilled water.

R3: Reagent 3

Purified Human Factor IXa, containing calcium, lyophilised. Reconstitute each vial with exactly 4 ml of distilled water.

Nota:

- The Human plasma used for the purification of Fibrinogen, Prothrombin, Protein S (R1), Factor X (R2A and R2B), APC and Factor IXa (R3) was tested and found negative for HIV antibodies, HBs Ag and HVC antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.
- Bovine Serum Albumin, used as a stabilizing factor, is prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no test may totally exclude the absence of infectious agents. As any product of bovine origin, this reagent R1 must be used with all the cautions required for handling a material potentially infectious.

REAGENTS AND MATERIAL REQUIRED BUT NOT SUPPLIED:

Reagents:

- Distilled Water; and Physiological saline.
- Normal Control Plasma (# 223201) and Abnormal Control Plasma (BIOPHEN Act. PC-r Control Plasma, # 223405) for Factor V Leiden.

Materials :

- Electromagnetic water bath or semi automatic or automatic clotting instruments.
- Chronometer.
- Calibrated pipettes of 50 and 100 µl.

CONSERVATION:

In their original package, and before any use, when stored at 2-8°C, the Hemoclot Factor V-L reagents are stable until the expiration date printed on the kit.

Nota : The stability studies at 30°C show that all the reagents can be shipped at room temperature without damage.

REAGENT PREPARATION AND STABILITY:

Preparation

Reagent 1: R1: Purified coagulation Factors:

Restore each vial with exactly 4 ml of distilled water. Let for 30 min at room temperature (18-25°C); mix gently until complete dissolution of the content (vortex). Homogenise before each use.

Reagent 2A: R2A: Purified Human Factor X in presence of cephalin:

Restore each vial with exactly 1 ml of distilled water. Let for 30 min at room temperature (18-25°C); mix gently until complete dissolution of the content (vortex). Homogenise before each use.

Reagent 2 B: R2B : Purified Human Factor X in presence of APC and cephalin:

Restore each vial with exactly 1 ml of distilled water. Let for 30 min at room temperature (18-25°C); mix gently until complete dissolution of the content (vortex). Homogenise before each use.

Reagent 3: R3 : Human Factor IXa, containing calcium.

Restore each vial with exactly 4 ml of distilled water. Let for 30 min at room temperature (18-25°C); mix gently until complete dissolution of the content (vortex); homogenise the content before each use.

Stability

Stability of the reconstituted reagents R1, R2A, R2B et R3, stored in their original package, is:

24 hours 2 – 8 °C	8 hours Room Temperature	1 month Deep frozen at -20°C or less
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Cautions:

In order to improve stability, reagents must be closed with their original screw cap following each use. Reagents must be handled with care, in order to avoid any contamination during use.

Nota:

- R1, R2A, R2B and R3 vials are closed under vacuum. Remove carefully the stopper, in order to avoid any lost of powder when opening the vials.
- If the reagents are frozen, thaw for 30 minutes at 37°C; mix gently (vortex) the thawed reagents before any use.
- According to the automated method used, the reagents can be reconstituted with volumes different from those recommended. In any case, the established reactive ratios (respective reagent concentrations in the reactive milieu and total volume) for each reagent must be strictly respected.
- Use only reagents from kits with a same lot number. Do not use reagents from kits with different lots when running the assay. Reagents R1, R2A, R2B and R3 are optimized for each lot of kits.

PREPARATION OF PLASMA:

Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), with great care, in order to avoid any activation. Sampling must be performed through a net venipuncture, and the first drops must be discarded.

- Within 2 hours, blood must be centrifuged at 3,000 g for 20 min at 18°C or below, and plasma decanted into a plastic tube, using a plastic pipette.
- Conservation of plasma:
 - Up to 8 hours at 20°C
 - Up to 24 hours at 2-8°C
 - Up to 1 month frozen at -20°C or below (before use, thaw for 15 min. in a water bath at 37°C).

PROCEDURE:

The HEMOCLOT Factor V-L kit is a clotting method, manual or automated. Adaptations on automates are available upon request. The assay is performed at 37°C, and the clotting time, triggered by R3 reagent, is measured.

Test plasma:

Tested plasma must be diluted 1/5 in physiological saline (9g/l sodium chloride, NaCl) or in Imidazole buffer.

Manual method

Assay	Without APC	With APC
Plasma diluted 1/5 in physiological saline	50µl	
Reagent R1	100µl	
Mix and incubate 1 minute at 37°C then introduce		
Reagent R2A or	50µl	
Reagent R2B		50µl
Mix and incubate 1 minute at 37°C then introduce		
Reagent R3, pre-incubated at 37°C, and stirred	100µl	
Record Clotting Times	CT1	CT2

The ratio CT2/CT1 must be calculated.

Automated methods:

Adaptations to the various analysers (STA-R, BCS, etc...) are available upon request. Refer to each specific adaptation and specific cautions for each instrument.

Note:

- In order to maintain the assay performances, the same respective proportions between reagent concentrations and volumes used, must be adhered to.
- Discard any sampling of plasma which has a "colour" different from the usual one.

QUALITY CONTROL:

Use of quality control plasmas, Normal or Abnormal for Factor V-L, allows validating the assay performances and the homogeneous reactivity of the HEMOCLOT Factor V-L kit, from run to run, when using a same lot of reagents. These controls are available with the following the references:

Biophen Normal Control Plasma: (#223201).

Biophen Act PC-r Control Plasma: (#223405).

LIMITATIONS OF THE PROCEDURE:

- The Clotting Times obtained are sensitive to the concentration of Factor V. Only the CT2/CT1 ratio allows evidencing the presence of Factor V-L. If the concentration of Factor V is decreased, both Clotting Times (CT1 and CT2) are increased proportionally, but the CT2/CT1 ratio remains usually valid. The assay can be performed in patients with LA, ACA, heparin therapy or dicoumarol treated. In presence of an abnormally prolonged CT1 (> 45 sec), confirming the diagnosis with another method is recommended. A factor V concentration ≥ 20% is required in the tested specimen for the right performance of the assay.
- Inappropriate specimen collection and plasma preparation may induce a consumption of Factors V and VIII: C, which can induce prolonged Clotting times for CT1 and CT2. Presence of activated clotting factors may shorten CT1 and CT2, and the CT2/CT1 ratio.
- Using of thawed reagents slightly prolong clotting times measured.
- For a same reagent lot and a same tested specimen, CT1 and CT2 may present variations according to the instrument used, and to the clot detection sensitivity adjustment. This might affect the CT2/CT1 ratio. Always validate the cut-off values used for normals and for patients carrying the Factor V mutation, and, if necessary, adjust them.

RESULTS:

The ratio of the Clotting Times obtained with or without APC, CT2/CT1, allows measuring the sensitivity of Factor V, in the tested specimen, to the action of Activated Protein C.

Normal plasma, containing normal Factor V, is sensitive to this action, and yields a ratio: **CT2/CT1 ≥ 2.00**

Plasmas from patients carrying the R506Q mutation of Factor V, i.e. containing Factor V Leiden, yield a ratio: **CT2/CT1 ≤ 1.80**.

Molecular biology allows confirming the diagnosis, and classifying patients as heterozygous or homozygous.

SPECIFIC PERFORMANCE CHARACTERISTICS:

Example of values obtained using the STA-R instrument:

Samples		N	Mean
Normal Plasmas	CT1	21	33.6 sec.
	CT2	21	74.2 sec.
	Ratio	21	2.22
Heterozygous Plasmas	CT1	8	36.3 sec.
	CT2	8	56.5 sec.
	Ratio	8	1.56

EXPECTED VALUES:

The incidence of Factor V-L (R506Q mutation) is variable according to the geographical area. It is higher than 15% in Scandinavian countries and of about 5% in Mediterranean countries. This mutation is absent in the Chinese or Japanese populations.

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