HEMOCLOT Quanti. V-L

Ref CK065K

IVD

Quantitative measurement of Factor V Leiden activity by testing its resistance to the action of Activated Protein C

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INTENDED USE:

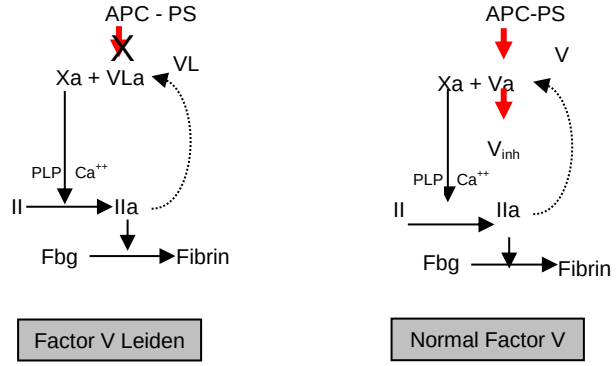
HEMOCLOT Quanti. V-L Kit is a clotting method for measuring the Factor V Leiden (Factor VL) activity in human citrated plasma, by its resistance to the action of Activated Protein C (APC) with the presence in excess of Protein S. The assay is performed in the presence of Activated Protein C (one single test for each patient). In the presence of APC, the prolongation of clotting time is an inverse relationship of the concentration of Factor VL (mutation R506Q). Normal Factor V is not measured.

SUMMARY AND EXPLANATION:

The Factor V Leiden is insensitive to the action of Activated Protein C. Its presence induces a prolonged coagulant activity in blood circulation (due to a prolonged survival of Factor Va activity), following activation of coagulation. Patients with the Factor VL (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 carry Factor V Leiden.

ASSAY PRINCIPLE:

The HEMOCLOT Quanti. V-L kit is a coagulation method performed on the diluted test plasma, supplemented with a constant and in excess amount of Activated Protein C and clotting factors. Clotting is triggered by purified Factor Xa in presence of Phospholipids and Calcium.
In the first step, the diluted plasma is mixed with purified clotting Factors (Prothrombin, Fibrinogen, Protein S and Activated Protein C (APC) (R1), in a constant and optimised concentration. In second step, the purified Factor Xa in presence of Phospholipids (PLP), also in a constant and optimised concentration, is added (R2). Clotting is initiated by addition of calcium (Ca^{2+}) and clotting time (CT) is then recorded. Clotting time observed is inversely proportional to the concentration of Factor VL in the sample. There is an inverse linear relationship, on a linear (CT)-logarithmic (% Factor VL) graph paper, between the Factor VL concentration and the corresponding clotting time.



REAGENTS:

HEMOCLOT Quanti. VL contains all the different reagents required for testing 3 series of 20 tests with the manual method.
R1: Reagent 1: Clotting mixture containing human Fibrinogen, human Prothrombin, Protein S at a constant concentration, optimised for the assay, and human Activated Protein C, lyophilised. It also contains heparin neutralizing substance. Reconstitute each vial with exactly **2 mL** of distilled water (3 vials of 2 ml).
R2: Reagent 2: Purified Human Factor Xa, containing rabbit brain Cephalin (phospholipids' source), lyophilised. Reconstitute each vial with exactly **1 mL** of distilled water (3 vials of 1 ml).

CAUTIONS AND WARNING:

- Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.
- Human plasma used for the purification of Fibrinogen, Prothrombin, Protein S, APC (R1), and Factor Xa (R2), was tested and found negative for HIV antibodies, HBs Ag and HVC antibodies. 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 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.
- R1 and R2 vials are closed under vacuum. Remove carefully the stopper, in order to avoid any lost of powder when opening the vials.
- Use only reagents from kits with the same lot number.
- The disposal of waste materials must be carried out according to current local regulations
- 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 AND STABILITY OF REAGENTS:

R1: **Reagent 1**: **Purified coagulation factors mixture:**
Restore each vial with exactly **2 mL** of distilled water. Shake thoroughly until complete dissolution. Let stand at room temperature (18-25°C) for 30 min, while shaking the vial from time to time. Homogenize well before each use.
Stability of reagent R1, provided that any contamination or evaporation is avoided, kept in its original vial or in a plastic tube closed:

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

R2: **Reagent 2**: **Human Factor Xa**, containing rabbit brain Cephalin.
Restore each vial with exactly **1 mL** of distilled water. Shake thoroughly until complete dissolution. Let stand at room temperature (18-25°C) for 30 min, while shaking the vial from time to time. Homogenize the content before each use.
Stability of R2, provided that any contamination or evaporation is avoided, kept in its original vial or in a plastic tube closed:

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

*: frozen reagents must be thawed rapidly at 37°C before use, shake gently. The thawing time should be adapted to the volume of product.

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.

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;
- Imidazole Buffer (Ref. AR021A/AR021K/AR021L).
- CaCl2 0.025M (Ref. AR001A/AR001K).
- Calibration plasmas, lyophilised, undiluted: BIOPHEN V-L Cal. (# 222401).
- Quality control plasmas: normal (BIOPHEN Normal Control Plasma - # 223201), or carrying the R506Q Factor V mutation (BIOPHEN Act. PC-r Control Plasma - # 223405).

Materials:

- Electromagnetic water bath or semi automatic or automatic clotting instruments.
- Chronometer.
- Calibrated pipettes.

SPECIMEN COLLECTION:

Preparation and storage of samples are performed as recommended by GEHT or NCCLS/CLSI.

- Samples:
  Human plasma obtained from Trisodium Citrate anticoagulated blood.
- Collection:
  Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) through a net venipuncture. The first tube must be discarded. The delay between the collections and the tests is ideally 1 to 2 hours and should not exceed 4 hours.
- Centrifugation:
  Use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2000 g at room temperature (18-25°C)
- Storage of plasma:
  - 4 hours at room temperature (18-25°C)
  - 2 months at -20°C.
  - 18 months at -70°C.

TEST PROCEDURE:

The HEMOCLOT Quanti V-L kit is a clotting method, manual or automated. Applications on automates are available upon request. The assay is performed **at 37°C**, and the clotting time, triggered by Factor Xa, Cephalin and calcium, is measured

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

Calibration curve:
The **BIOPHEN V-L Cal.** kit **(ref 222401)** can be used, composed of 3 levels of undiluted plasma calibrators with a well defined Factor VL concentration. After reconstitution according to the pack insert of the kit (refer 222401), the lot specific

concentration of Factor VL "C" is obtained with a 1:20 dilution of each calibrator. The "2C" concentration (of about 100%) is obtained with a 1:10 dilution of calibrator 3.
This kit of calibrators covers the range from about 10% to 100% Factor VL. The exact concentration "C" in (% Factor VL) is indicated on the lot specific flyer provided in the kit box.

Tested plasma:
Tested plasma must be diluted **1:20** in **Imidazole** buffer. It should be tested within **2 hours** when stored at room temperature (18-25°C).

Assay (Manual method):
In a plastic test tube, or in a reaction cuvette, introduce:

Assay	Manual method
Reagent **R1**	100µl
Plasma diluted **1:20** or calibrator	100µl
Mix and incubate for exactly 1 minute at 37°C then introduce	
Reagent **R2**	50µl
Mix and incubate for exactly 1 minute at 37°C then introduce	
$CaCl_2$ 0.025M pre-incubated at 37°C	100µl
Record Clotting Times	**CT**

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 volumes used, must be adhered to, in order to maintain the assay performances.

QUALITY CONTROL:

Using suitable commercially available quality control plasmas, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents.
At least one quality control at each level in each series, as per good laboratory practice should be included to valid it. 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.

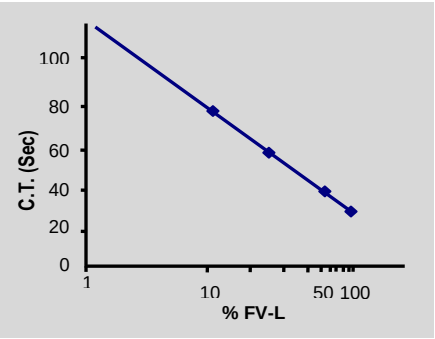
RESULTS:

Create the calibration curve and plot on abscissa (using a logarithmic scale) the Factor VL concentrations **(%)** and on ordinates (using a linear scale) the corresponding clotting times **(sec)**. From the measured clot time interpolate directly the corresponding Factor VL concentration for the tested plasma.

The Clotting Time, obtained in presence of APC (constant and in excess) allows measuring, in the tested specimen, the Factor V sensitivity to the action of activated Protein C. The expected value for Normal plasma, containing normal Factor V, fully sensitive to this action, is expected **< 10% F V-L.** The expected value for a plasma with low sensitivity to the activated protein C (carrying mutated Factor VL), is between **25 and 75% FV-L (heterozygote)** or **> 75% FV-L (homozygote).**
Factor VL concentrations observed can be out of these ranges depending on the actual Factor V clotting activity in the tested plasma. Results interpretation can be optimised by comparing Factor VL concentration to the Factor V clotting activity (ratio around 1.0 for homozygotes, around 0.5 for heterozygotes, and <0.1 for normal).
By definition, the twenty fold dilution (1:20) of a pool of plasmas from heterozygous patients carrying the R506Q mutation of Factor V, i.e. containing Factor VL and presenting a low sensitivity to activated protein C, corresponds to a concentration of 50% of Factor VL.
Molecular Biology only allows confirmation of the patient classification as heterozygous or homozygous for Factor V Leiden mutation.
This method is very sensitive to measure the presence or absence of Factor VL. It is the very convenient assay for quantitating the Factor VL associated clotting activity.

EXAMPLE OF CALIBRATION CURVE:

The calibration curve below, obtained on manual method is indicated as an example only. The calibration curve generated for the series of measures performed must be used.



LIMITATION:

- The Clotting Times obtained are sensitive to the concentration of Factor V; Factor V deficiency (<25%) in a patient carrying the Factor VL mutation can induce wrong results.
- Inappropriate specimen collection and plasma preparation, may induce a consumption of Factor V, which can induce prolonged Clotting times.
- Presence of activated clotting factors may shorten CT.
- The assay can be performed for patients under Heparin (up to 1 IU/ml) or anti-vitamin K (AVK) therapy.
- Assay of patients with lupus anticoagulant is not recommended as the interference was not extensively evaluated in the assay.
- The possible interference of mutations such has FV Cambridge or FV Hong Kong was not evaluated in the assay.

EXPECTED VALUE:

The incidence of Factor VL (R506Q mutation) is variable according to the geographical area. It is about 5-6% in the U.S. and Canada. 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.
A normal range study performed using the Hemoclot Quanti V-L kit resulted in range 0 to 10% Factor V-L (manual method: N=30, Mean= 1.9% Factor V-L SD = 2.4; STAR: N=89 Mean= 2.4% FV-L SD=2.3). Each laboratory should verify and validate the expected ranges in the exact working conditions.

PERFORMANCE CHARACTERISTICS:

- Example of reproducibility obtained for 2 plasmas at different Factor VL concentrations, using the KC10, STAR or WATER BATH instrument:

KC10	Intra Assay CV%			Inter Assay CV%		
Sample	On CT (sec)	On %FVL	N	On CT (sec)	On %FVL	N
Sample 1 (100% FVL)	4.4%	5.9%	10	2.2%	3.3%	10
Sample 2 (50% FVL)	4.2%	8.2%	10	2.3%	4.7%	10

Instrument	Sample	Inter Assay CV%		
		On CT (sec)	On %FVL	N
KC10	Sample 1 (25% FVL)	7.3%	17.4%	5
	Sample 2 (10% FVL)	6%	29.1%	5
STAR	Sample 1 (25% FVL)	5.2%	13.1%	10
	Sample 2 (10% FVL)	5.6%	23.7%	10
WATER BATH	Sample 1 (25% FVL)	2.7%	10.0%	5
	Sample 2 (10% FVL)	5.6%	15.2%	5

- The HEMOCLOT Quanti. V-L assay shows good consistency with Coatest APCr kit (Chromogenix), as shown from data combined from 4 studies:

All Sites		Coatest APCr	
		Normal	Abnormal
Hemoclot FVL-Q	Normal	108	7
	Abnormal	1	70
	Inconclusive*	2	1
Agreement Co-positivity Co-negativity Sample Size		94.18% 97.30% 89.74% 189	

*Outside of the decision range of the assay, between 10% and 25% FV-L.

- The HEMOCLOT Quanti. V-L assay shows good consistency with Molecular Biology results:

		Molecular Biology Diagnosis	
		Normal	Abnormal
Hemoclot FVL-Quanti	Normal	15	0
	Abnormal	0	6
Agreement Co-positivity Co-negativity Sample Size		100% 100% 100% 21	

- The HEMOCLOT Quanti. V-L kit shows a good homogeneity with the kit HEMOCLOT FV-L (HYPHEN BioMed), on STAR:

patients (R506Q mutation): Mean [Min-Max]	Ratio (HEMOCLOT FVL)	%FVL (HEMOCLOT Quanti. VL, STAR)
Normal (N=21)	>2.0 [2.05-2.44]	<5% [0-9%]
Heterozygotes (N=37)	1.72 [1.57-1.80]	51% [27-69%]
Heterozygotes (treatment AVK)(N=20)	1.73 [1.56-1.84]	52% [34-75%]
Homozygotes (N=16)	1.40 [1.24-1.44]	92% [73-118%]

Study realized outside USA.

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SYMBOLS:

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