HEMOCLOT™ Quanti. VL

CE

IVD

REF CK065K

R1 3 x 2 mL

R2 3 x 1 mL

Clotting method for the determination of Factor V Leiden activity.

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INTENDED USE:

HEMOCLOT™ Quanti. VL kit is a clotting method for the *in vitro* quantitative determination of Facteur V Leiden (FV-L), by measuring its resistance to the inactivation by Activated Protein C (APC), in the presence of Protein S, on human citrated plasma, using manual or automated method.

SUMMARY AND EXPLANATION:

Technical[1,2]***:***

Activated Protein C plays a role of regulator in the coagulation process by specifically inactivating activated Factors V (Va) and VIII (VIIIa), in the presence of co-factors. The resistance phenomenon to Activated Protein C (APC) is due in more than 90% of cases to the R506Q mutation of Factor V called "Factor V Leiden". This mutation in Factor V exon 10 (1691 G → A) substitutes arginine at position 506 by glutamine, prevents this site cleavage by APC.

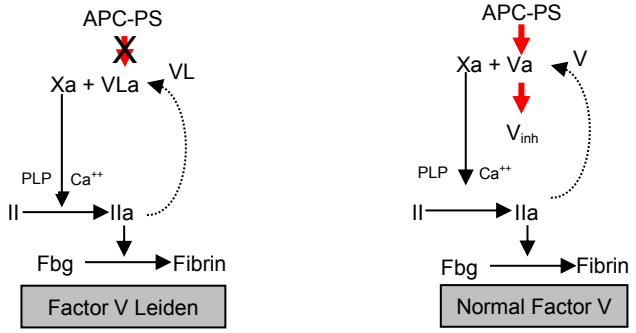
Clinical[2,3,4]***:***

FV-L mutation is the most common hereditary thrombophilia risk factor. Its prevalence is about 5% in Caucasian populations. Patients carrying FV-L mutation have an increased risk of venous thrombosis, 3 to 7 fold in heterozygotes and up to 80 fold in homozygotes.

This genetic anomaly can be evidenced by clotting assay in the presence or absence of APC.

PRINCIPLE:

HEMOCLOT™ Quanti. VL method is a coagulation assay for FV-L quantification by measuring its sensitivity to inactivation by APC, in the presence of PS. The coagulation test is performed in the presence of an excess of APC and coagulation factors (Prothrombin, Fibrinogen and PS). Coagulation is triggered by purified factor Xa (in constant and optimized concentration) in the presence of phospholipids and calcium. The measured clotting time (CT) is inversely proportional to FV-L concentration present in the tested specimen. Normal factor V is not measured.



REAGENTS:

R1 **Clotting mixture**, lyophilized. Contains human Fibrinogen, human Prothrombin, PS at a constant concentration, optimized for the assay, human APC, an heparin neutralizing substance, BSA and stabilizers.
3 vials of 2 mL.

R2 **Purified Human Factor Xa**, in constant and optimized concentration for the test, lyophilized. Contains rabbit brain cephalin (phospholipids' source), BSA and stabilizers.
3 vials of 1 mL.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R1 Reconstitute the contents of each vial with exactly **2 mL of distilled water.**
Shake vigorously until complete dissolution while avoiding formation of foam and load it directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

R2 Reconstitute the contents of each vial with exactly **1 mL of distilled water.**
Shake vigorously until complete dissolution while avoiding formation of foam and load it directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R1 R2 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **24 hours** at 2-8°C.
- **12 hours** at room temperature (18-25°C).
- **1 month** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific application.**

*Thaw only once, as rapidly as possible at 37°C and use immediately.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole Buffer (AR021B/AR021K/AR021L/AR021M/AR021N).
- $CaCl_2$ 0.025 M (AR001B/AR001K/AR001L).
- Specific calibrators and controls, such as:

Product name	Refernce
BIOPHEN™ V-L Plasma Calibrator	222401
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ V-L Control Plasma	223405

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

Materials:

- Electromagnetic water bath or semi-automatic or automatic clotting instruments.
- Chronometer, calibrated pipettes, silicon glass or plastic test tubes.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[5] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[5].

PROCEDURE:

The kit can be used in manual or automated method. Perform the test at 37°C and the clotting time, triggered by addition of Calcium, is measured.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. For the calibration curve, dilute the calibrators in imidazole buffer as described below in order to establish the calibration range ("C" defines the concentration of FV-L, corresponding to the **1:20** dilution of the calibrator):

Calibrator	C1	C2	C3	C3 (2C)
FV-L (%) **about**	10	25	50	100
Volume of calibrator	50µL of C1	50µL of C2	50µL of C3	100µL of C3
Volume of Imidazole buffer	950µL	950µL	950µL	900µL

2. Dilute the specimens in Imidazole buffer, as described in the table below:

Specimens	Reference	Dilution
Controls	223201 / 223405	**1:20**
Specimen	n.a.	**1:20**

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. Introduce into a reaction cuvette, silicon glass or plastic test tube incubated at **37°C**:

Reagents	Volume
R1 Clotting Mixture	100 µL
Diluted calibrators, specimens or controls	100 µL
Mix and incubate at **37°C** for **exactly 1 minute**	
R2 Human Factor Xa	50 µL
Mix and incubate at **37°C** for **exactly 1 minute,** then introduce (starting the stop-watch) :	
$CaCl_2$ 0.025M preincubated at **37°C**	100µL
Record the exact clotting time (CT, sec).	

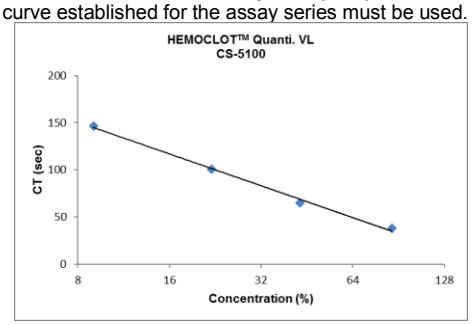
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The HEMOCLOT™ Quanti. VL assay can be calibrated for the assay of FV-L. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

- The calibration range is about 10 to 100% (on CS-series).

The calibration curve shown below is given by way of example only. The calibration curve established for the assay series must be used.



QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve lin-log, with the clotting time (sec) along the Y-axis and the FV-L concentration, expressed as %, along the X-axis.
- The concentration of FV-L (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- The clotting times obtained are sensitive to the Factor V concentration; Factor V deficiency (<25%) in a patient carrying FV-L 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 vitamin K antagonist (VKA) 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 VALUES:

FV-L concentration for normal plasma is **< 10%.** However, each laboratory has to determine its own normal range.
The expected value in a plasma with R506Q mutation is, for an heterozygote, usually between **25 and 75%** and for an homozygote **> 75%.**
Results interpretation can be optimised by comparing FV-L concentration to the Factor V clotting activity (ratio around 1.0 for homozygotes, around 0.5 for heterozygotes, and <0.1 for normals).
Molecular Biology only allows confirmation of the classification as heterozygous or homozygous for FV-L mutation.

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used (<2% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 5 to 120% of FV-L on Sysmex CS-series).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 3 repetitions within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assays			
	n	Mean	CV%	SD	n	Mean	CV%	SD
Control 1	40	10.8	2.9	0.3	30	11.1	2.8	0.3
Control 2	40	43.1	2.0	0.8	30	44.7	2.4	1.1

- Correlation with reference method (COATEST™ APC™ Resistance V on ACL Top vs HEMOCLOT™ Quanti. V-L on Sysmex CS-5100) : 99% agreement (n = 116).
- Interferences:

No interference, on the instrument Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	Bilirubin (Free)	Bilirubin (Conjuguated)
1000 mg/dL	30 mg/dL	60 mg/dL
Intralipids	**Heparin (UFH/LMWH)**	**Dabigatran**
1000 mg/dL	2 IU/mL	50 ng/mL

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

REFERENCES:

1. Bertina R.M. *et al*. Mutation in blood coagulation factor V associated with Resistance to Activated protein C. Nature. 1994.
2. Segers K. *et al.* Coagulation factor V and thrombophilia: Background and mechanisms. Thromb Haemost. 2007.
3. Kadauke S. *et al.* Activated protein C resistance testing for factor V Leiden. American Journal of Hematology. 2014.
4. Freyburger G. and Labrouche S. Facteur V Leiden (VL) et résistance à la protéine C activée (PCA), facteur II Leiden (G20210 G>A), aspects physiopathologiques et stratégies diagnostiques. Spectra Biologie. 2007.
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.