

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k083729

B. Purpose for Submission:

Bundled submission for clearance of a new Factor V-Leiden (FVL) assay and its associated calibrators and control, and modification for a currently cleared coagulation control (k043451).

C. Measurand:

Factor V-Leiden

D. Type of Test:

Clotting Assay

E. Applicant:

ANIARA DIAGNOSTICA, LLC

F. Proprietary and Established Names:

Hemoclot Quanti V-L
Factor V-L Calibrator
Biophen V-L Cal (Undiluted)
Biophen ACT PC-r Control Plasma
Biophen Normal Control

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
GGW, Test Partial Thrombin Time GGN, Plasma, Coagulation Control JIT, Calibrator, Secondary	Class II	21 CFR 864.7925 21 CFR 864.542521 CFR 862.1150	81 Hematology

H. Intended Use:

1. Intended use(s):

HEMOCLOT Quanti V-L is a clotting method for measuring the Factor V Leiden (FVL) activity in human citrated plasma, by its resistance to the action of Activated Protein C (APC).

Factor V-L Calibrator is lyophilized, pre-diluted (1:20) human plasmas, at defined Factor V-Leiden (FV-L) concentrations, for the calibration of Factor V-L activity quantitative clotting assay on human citrated plasma, using the HEMOCLOT Quanti. V-L Kit (ref CK065K).

BIOPHEN V-L CAL (Undiluted) is lyophilized, undiluted human plasmas, at defined Factor V-Leiden (FV-L) concentrations for the calibration curve of Factor

V-L activity quantitative clotting assay on human citrated plasma, using HEMOCLOT Quanti V-L Kit (ref CK065K).

BIOPHEN Act PC-r Control Plasma kit contains human plasma, presenting an activated Protein C Resistance (APC-R), usually correlated with the genetic mutation of Factor V R506Q. This plasma is used as quality control plasma for the testing of Activated Protein C Resistance (APC-R).

BIOPHEN Normal Control Plasma is a set of 12 vials of normal citrated human plasma for the quality control of some coagulation factors (ATIII, Protein C, aPC resistance FV Leiden, Lupus Anticoagulant).

2. Indication(s) for use:
Same as Intended Use.
3. Special conditions for use statement(s):
For Prescription use only.
4. Special instrument requirements:
Sigma Diagnostics KC 1A (k955724)
Sigma Diagnostics AMAX 400 (k972260)
American Bioproducts STA (k942117)
American Bioproducts STA-R (k082675)
Manual (Water Bath)

I. Device Description:

HEMOCLOT Quanti V-L is an in vitro diagnostic kit containing two reagents to measure the Factor V Leiden concentration in citrated plasma, by its resistance to the Activated Protein C. Reagent 1 is a clotting mixture containing human Fibrinogen, human Prothrombin, and Protein S at a constant concentration, optimized for the assay, and human Activated Protein C, lyophilized. It also contains a heparin neutralizing substance. Reagent 2 is Purified Human Factor Xa, containing rabbit brain phospholipids (cephalin), lyophilized. Calibrators and controls are required and sold separately.

FVL Calibrator kit: 12 vials (3 sets of 4 vials) of 1 mL of prediluted human plasma (1:20) at different concentrations of FV-L, ranging from about 10% to 100%.

Biophen V-L Cal (Undiluted) kit: 9 vials (3 sets of 3 vials) of 0.5 mL of undiluted human plasma at different concentrations of FV-L, to cover the assay range, from about 10% to 100%. (3 vials for each concentration, 4 points for the calibration curve).

Act. PC-r Control Plasma kit: 12 vials of 0.5 mL of human plasma, presenting an APC-R, citrated and lyophilized.

BIOPHEN Normal Control Plasma: 12 vials of 1 mL of normal citrated human plasma, lyophilized.

J. Substantial Equivalence Information:

	New Device	Predicate Device
	HEMOCLOT Quanti V-L, Factor V-L Calibrator, Biophen V-L CAL (undiluted), Biophen Act PC-r Control Plasma, Biophen Normal Control Plasma	COATEST APC Resistance V (K963111). Control Plasmas Level 1 and Level 2 are included in this device kit.
	Similarities	
Intended Use	Measurement of Factor V Leiden activity in human citrated plasma by its resistance to the action of Activated Protein C.	Same
Assay Type	Clotting Method	Same
Test Sample	Human Citrated Plasma	Same
Assay Principle	Measurement of Factor V Leiden coagulation activity based on insensitivity of Factor V Leiden to the action of Activated Protein C.	Same
Stability of Unopened Reagents	Stable at 2-8°C until expiry date	Same
Controls, Normal	Citrated human plasma, lyophilized, to provide quality control in the normal range. APC V Ratio > 2.	Same
Controls, Abnormal (APC Resistant)	Citrated human plasma, lyophilized, to provide quality control for APC resistant range of the assay. APC V Ratio < 1.80	Same
	Differences	
Assay Principle	Assay is performed in the presence of Activated Protein C and Protein S (one single test for each patient). In the presence of APC, prolongation of clotting time is inversely related to the amount of Factor V Leiden. Normal Factor V is not measured. APC resistance due to Factor V Leiden is indicated when the %FVL value is above or equal to the cut-off value.	Assay is performed in the presence and absence of Activated Protein C (two tests for each patient). In the presence of APC, prolongation of clotting time is directly related to the concentration of Normal Factor V, and inversely related to the amount of Factor V Leiden. APC resistance due to Factor V Leiden is indicated when the APC-V ratio is below or equal to the cut-off value.
Assay Calibration	Assay of calibrator plasma at defined Factor V Leiden concentrations. Calibrator plasmas available in prediluted (<u>FVL Calibrator</u>) and undiluted (<u>BIOPHEN V-L Cal</u>) form, lyophilized.	Not Applicable.

K. Standard/Guidance Document Referenced (if applicable):

STANDARDS

Title and Reference Number

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Other Standards

GUIDANCE

Document Title	Office	Division	Web Page
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L. Test Principle:

The Hemoclot Quanti V-L kit is a clotting method triggered by purified Factor Xa, containing Phospholipids, and Calcium, and is performed on diluted test plasma, in presence of Activated Protein C and Protein S. In the first step, diluted plasma is mixed with purified clotting Factors [clotting mixture containing Prothrombin, Fibrinogen, Protein S and Activated Protein C (APC)], in a constant and optimized concentration. Then, the purified Factor Xa in the presence of Phospholipids (PLP) is added. Clotting is initiated by the addition of Calcium (Ca²⁺). The clotting time is then recorded. Clotting time measured is inversely proportional to the concentration of factor V-L. There is an inverse linear relationship, on a lin-log graph paper, between the factor V-L concentration and the corresponding clotting time.

FVL Calibrator and Biophen V-L Cal (Undiluted) are two calibrators that may be used with the HEMOCLOT Quanti.V-L kit. Biophen V-L CAL (Undiluted) is lyophilized undiluted human plasma containing different concentrations of FV-L, to cover the assay range, from about 10% to 100%. FVL Calibrator is lyophilized pre-diluted human plasma (1:20) at different concentrations of FV-L, to cover the assay range, from about 10% to 100%.

Biophen Normal Control Plasma and Biophen Act. PC-r Control Plasma are quality control plasmas which may be used with the kit.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Two lots of Hemoclot Quanti V-L were analyzed for inter-assay precision. In one study the assay was performed with the waterbath, over four days using the internal reference calibrator at 4 different concentrations (10%, 25%, 50%, 100%) of FVL. Normal Control and the Biophen APCr control were used as controls. In another study using the waterbath, 9 vials of reagent was assayed at the 100% FVL level by several technicians. Inter-assay precision was also demonstrated using the KC10 analyzer by performing 10 assay runs over

several days using 2 technicians. The calibration curve was run, which represents the entire range of the assay, 10%-100% FVL.

Intra-assay precision was demonstrated on the waterbath by assaying 10 reagent vials from the same lot at the 100% FVL Level, in the single run.

All analyses yielded CV<6%.

Additional precision testing was performed at the medical decision points (10%, 25%) on the KC10 (10 runs), STA-R (10 runs), and waterbath (5 runs). Two samples were assayed over 3 days using 3 technicians. For each run a reference calibration curve and Normal Control and Biophen APCr was performed.

b. Linearity/assay reportable range:

The linearity of the Hemoclot Quanti V-L assay was evaluated by use of FVL Calibrator plasmas at 4 different concentrations of FVL, 10%, 25%, 50%, and 100%. The linearity of the assay was validated on the waterbath and several commercial analyzers (KC10, STA, STAR and Amax 400). The assay is linear between 10% and 100% FVL, $r^2 > 0.98$. The linearity of the assay below 10% is not determined. Any value below 10% is considered normal. Values between 25% and 75% are expected for heterozygous plasmas. Values >75% are expected for homozygous plasmas. Values between 10 -25% are considered inconclusive.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The Internal Reference Standard used for assigning values to the calibrators and controls is traceable to pooled normal and APCr human plasmas, controlled for normal Factor V content against commercially available reference plasma pools, and verified against an NIBSC standard for normal Factor V (SSC/ISTH Secondary Coagulation Standard).

The calibration curve for the Hemoclot Quanti V-L kit is established with either Biophen V-L cal (undiluted) or Factor V-L Calibrator kits. By definition, normal plasma contains 0% FVL and heterozygous plasma, from patients carrying the R506Q mutation, contains 50% FVL. For the Biophen V-L cal (undiluted) kit, calibrators are made from mixtures of pooled heterozygous plasmas and pooled normal plasmas to yield calibration plasmas at concentrations of 10%, 25% and 50%. For the Factor V-L Calibrator kit, calibrators are made from mixtures of pooled heterozygous plasmas and pooled normal plasmas and diluted appropriately to yield calibration plasmas at concentrations of 10%, 25%, 50% and 100% FVL, ready to use with the Hemoclot Quanti V-L kit. The concentration of each calibrator level is accurately defined, against an internal reference standard, as the mean value from at least 10 vials tested in the same series by manual and/or automated methods, using the Hemoclot Quanti V-L Assay.

Biophen Act. PC-r Control Plasma and Biophen Normal Control Plasma (510k #043451) may be used for quality control with the Hemoclot Quanti V-L kit. Biophen Act PC-r Control Plasma is derived from pooled heterozygous plasma, from patients carrying the R506Q mutation, and contains about 50% FVL. Biophen Normal Control Plasma is derived from pooled normal plasmas and contains about 0% FVL. The FVL concentration of each control is accurately defined, against an internal reference standard, as the mean value of at least 3 vials tested in at least 3 independent series (i.e., at least 9 vials tested) by manual and/or automated methods, using the Hemoclot Quanti V-L Assay.

Stability: Reconstituted reagents are stable for 24 hrs at 2-8° C or 8 hrs at room temperature (18-25°C). Overheating studies support 30 month expiration dating for lyophilized reagents stored at 2-8°C.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

The specificity of the assay was demonstrated by analyzing factor deficient plasmas. Results from Factor deficient plasmas (V, X, II, PS, PC) showed no interference. Since the assay can be performed in patients with heparin therapy (up to 1 IU/mL), plasma samples spiked with heparin were tested and showed no significant interference as the result of a heparin neutralizing substance in Reagent 1. To determine whether the assay can be performed in patients with Anti-Vitamin K (AVK) therapy, plasma samples from APCr patients with AVK therapy were tested and yielded expected values.

f. *Assay cut-off:*

The normal cut-off range was determined and validated by analyzing normal patient plasmas and aPCr patient plasmas, carrying R506Q mutation (FVL) identified with molecular technology as Heterozygous (HTZ) or Homozygous (HMZ). Analyses were conducted on water bath, KC10 and STAR analyzers. Normal plasma is expected to measure <10%FVL. Plasmas from patients with Factor V Leiden are expected to measure between 25% and 75% FVL (heterozygous) or >75% (homozygous).

Using the Hemoclot Quanti V-L method, the normal plasma range was determined by analyzing 20 normal plasma samples from a French blood bank on three different instruments with normal and APCr Controls and a selection of ActPCr plasma samples. To verify the cut-off, 42 plasma samples from FVL/HTZ patients out of therapy, 20 plasma samples from FVL/HTZ patients under Anti Vitamin K (AVK) therapy and 18 plasma samples from FVL/HMZ patients were analyzed. Results were acceptable with normal samples measuring <10% FVL, 25% and 75% FVL for FVL/HTZ and >75% FVL for FVL/HMZ samples.

2. Comparison studies:

a. *Method comparison with predicate device:*

A four site (two in the US and two in Europe) comparison study was

conducted. 189 plasma samples were analyzed using the test (Hemoclot Quanti V-L) and predicate (Coatest APCr) method. Samples were selected from patients who were previously tested for coagulation disorders and represented both normal and APC-Resistant populations (about 60% of the samples were from normal patients and 40% from APCr patients). Patient plasma samples were selected to cover the range of the assay. Overall, there was 94% agreement between the two methods for determining APC resistance due to the Factor V Leiden mutation. In one study, samples were identified as heterozygous (25), homozygous (11), or normal (27) with regard to FVL mutation using molecular biology methods. There was 97% agreement between the results of test method and the molecular biology diagnosis with regard to APCr resistance.

All Sites		Predicate	
		Normal	Abnormal
Hemoclot Quanti V-L	Normal	108	7
	Abnormal	1	70
	Inconclusive*	2	1

*Outside the range of the Hemoclot Quanti V-L

Percent Total Agreement	94.18% (178/189)
Percent Positive Agreement	89.74% (70/78)
Percent Negative Agreement	97.30% (108/111)

- b. *Matrix comparison:*
Not applicable.
- 3. Clinical studies:
 - a. *Clinical Sensitivity:*
Not applicable.
 - b. *Clinical specificity:*
Not applicable.
 - c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.
- 4. Clinical cut-off:
Not applicable.
- 5. Expected values/Reference range:
Normal plasma is expected to measure <10%FVL. Plasmas from patients with Factor V Leiden are expected to measure between 25% and 75% FVL (heterozygous) or >75% (homozygous).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.