January 12, 2018

Roche Molecular Systems, Inc.
Pooja Shah
Sr. Regulatory Affairs Specialist
4300 Hacienda Drive
Pleasanton, California 94588

Re: K172913
Trade/Device Name: cobas® Factor II and Factor V Test
Regulation Number: 21 CFR 864.7280
Regulation Name: Factor V Leiden DNA mutation detection systems
Regulatory Class: Class II
Product Codes: NPR, NPQ
Dated: December 13, 2017
Received: December 14, 2017

Dear Pooja Shah:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR

Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Leonthena R. Carrington -S

Lea Carrington
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K172913

Device Name
cobas® Factor II and Factor V Test

Indications for Use *(Describe)*
The cobas® Factor II and Factor V Test is an in vitro diagnostic device that uses real-time PCR for the detection and genotyping of the Factor II (Prothrombin) G20210A mutation and the Factor V Leiden G1691A mutation in genomic DNA obtained from K2EDTA whole blood specimens as an aid in diagnosis of patients with suspected thrombophilia. The cobas® Factor II and Factor V Test and the cobas z 480 analyzer are used together for automated amplification and detection.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Attachment 3. 510k Summary

cobas® Factor II and Factor V Test
510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Molecular Systems, Inc.
Address	4300 Hacienda Drive, Pleasanton, CA 94588-2722 USA
Contact	Pooja Shah Phone: 925-596-8342 FAX: 925-225-0207 Email: pooja.shah.ps1@roche.com
Date Prepared	September 13th 2017
Proprietary Name	**cobas**® Factor II and Factor V Test
Common Name	N/A
Classification Name	Factor V Leiden DNA mutation detection system
Product Codes	NPR,NPQ 21 CFR 864.7280
Predicate Devices	K033612 & K033607
Establishment Registration	2243471 & 3004141078

1. DEVICE DESCRIPTION

The **cobas**® Factor II and Factor V Test is a real-time polymerase chain reaction (PCR) test for the qualitative detection and genotyping of a single point mutation in the human Factor V gene (G to A at position 1691, referred to as the Factor V Leiden mutation) and a single point mutation in the human Factor II gene (G to A at position 20210), in genomic DNA isolated from peripheral whole blood, as an aid in diagnosis of patients with suspected thrombophilia.

DNA is extracted offline from whole blood specimens collected in K2EDTA tubes. The user-selected DNA extraction method must provide DNA of sufficient concentration. Automated real-time PCR is then performed on the **cobas** z 480 analyzer. The Factor II and Factor V mutations are detected simultaneously in the same real-time PCR reaction. Depending upon the test order, results for one or both mutations are reported for each DNA sample.

The assay consists of one reagent kit and a system platform. The reagent kit provides the necessary reagents and controls to perform automated real-time PCR amplification and detection. The system platform consists of a real-time PCR thermal cycler (**cobas** z 480 analyzer) and a control unit (**cobas**® 4800 System CU). The amplification reactions generate a Factor II specific amplicon and a Factor V specific amplicon in all samples, and utilize four fluorescent-dye labeled oligonucleotide probes for detection of the Factor II and Factor V genotypes.

2. INDICATIONS FOR USE

The **cobas**® Factor II and Factor V Test is an in vitro diagnostic device that uses real-time PCR for the detection and genotyping of the Factor II (Prothrombin) G20210A mutation and the Factor V Leiden mutation G1691A in genomic DNA obtained from K2EDTA whole blood specimens as an aid in diagnosis of patients with suspected thrombophilia. The **cobas**® Factor II and Factor V Test and **cobas** z 480 analyzer are used together for automated amplification and detection.

3. TECHNOLOGICAL CHARACTERISTICS

The **cobas**® Factor II and Factor V Test has the same general intended use, technological characteristics and principles of operation as the two predicate devices. A substantial equivalence table comparing the similarities and differences between the **cobas**® Factor II and Factor V Test and its predicate devices is provided in Table 1

Table 1: Similarities and Differences between the cobas® Factor II and Factor V Test and the Predicate Device

	Submitted Device: cobas® Factor II and Factor V Test	**Predicate Device: Roche LightCycler® Factor II (Prothrombin) Test K033612**	**Predicate Device: Roche LightCycler® Factor V Leiden Test K033607**
Intended Use	The **cobas**® Factor II and Factor V Test is an *in vitro* diagnostic device that uses real-time PCR for the detection and genotyping of the Factor II (Prothrombin) G20210A mutation and the Factor V Leiden mutation in genomic DNA obtained from K_2EDTA whole blood specimens as an aid in diagnosis of patients with suspected thrombophilia. The test is performed on the **cobas z** 480 analyzer for automated amplification and detection.	Same as the Submitted device except the predicate device was cleared on the LightCycler® 1.2 Instrument.	Same as the Submitted device except the predicate device was cleared on the LightCycler® 1.2 Instrument.
Type of Test	Genotyping Test	Same	Same
Target of Detection	Single nucleotide polymorphism	Same	Same
Indication for Use	Aid in the diagnosis of patients with suspected thrombophilia	Same	Same
Intended User	Health Care Professional	Same	Same
Specimen Type	Purified DNA from human blood samples.	Same	Same
Technological Detection Principles	Real-time PCR test for simultaneous amplification of two targets and detection of specific SNPs in the PCR amplified DNA sequences (multiplex system).	Real-time PCR test for amplification of one target and detection of specific SNP in PCR-amplified DNA sequences	Real-time PCR test for amplification of one target and detection of specific SNP in PCR-amplified DNA sequences
Sample Preparation	DNA extraction and purification from whole blood, performed off-line	Same	Same

	Submitted Device: cobas® Factor II and Factor V Test	**Predicate Device: Roche LightCycler® Factor II (Prothrombin) Test K033612**	**Predicate Device: Roche LightCycler® Factor V Leiden Test K033607**
Oligonucleotide probes and primers	Specific for Factor V G1691A, Factor II G20210A and the wild type Factor II and Factor V sequences	Specific for Factor II G20210A and Factor II wild type sequences.	Specific for Factor V Leiden G1691A and Factor V wild type sequences
Detection Chemistry	Fluorogenic detection of SNPs in each PCR amplification product by allele-specific cleavage of TaqMan probes	Fluorogenic detection of SNP in PCR amplification product by melting curve analysis	Fluorogenic detection of SNP in PCR amplification product by melting curve analysis
Analytical Sensitivity	< 50 allele copies (0.01 ng input DNA/reaction)	Approximately 50 allele copies per reaction	Approximately 50 allele copies per reaction
Instrument	**cobas**® 4800 System	Roche LightCycler® version 1.2	Roche LightCycler® version 1.2
Controls used	Endogenous Internal control (each gene is internal control for other gene), plus external positive and negative controls required in each run.	External positive and negative controls required in each run.	External positive and negative controls required in each run.
Reference Method	Bi-directional Sanger sequencing	Sanger Sequencing	Sanger Sequencing

4. NON-CLINICAL PERFORMANCE EVALUATION

4.1. Analytical sensitivity (Lower Limit)

To determine the minimum input of genomic DNA necessary to yield correct Factor II and Factor V genotype results, genomic DNA was isolated from three K_2EDTA whole blood samples (Factor II heterozygous, Factor V heterozygous, Factor V homozygous mutant) and one cell line (Factor II homozygous mutant), using three different commercial DNA isolation methods for the whole blood samples, and one method for the cell line. Each genomic DNA sample was tested with the **cobas**® Factor II and Factor V Test at 10 concentrations: undiluted (which varied between 6 and 38 ng/µL) and nine serial dilutions from 1.0 to 0.0001 ng/µL. Each dilution was tested with 24 replicates with each of two kit lots, for a total of 48 replicates per concentration per genomic DNA sample. The undiluted genomic DNA samples were tested with six replicates with each kit lot, for a total of 12 replicates per genomic DNA sample.

The rate of correct Factor II and Factor V genotype results across all four samples, all three DNA isolation methods and both kit lots was 98% at 0.01 ng/µL and 100% at all higher concentrations (Table 2). The **cobas®** Factor II and Factor V Test is designed to yield Invalid results if the input DNA concentration is too low. There were no incorrect genotype results in the study. The limit of detection is 0.01 ng/µL, which is 10 times lower than the lowest recommended DNA input concentration.

Table 2: Analytical sensitivity of the cobas® Factor II and Factor V Test

Concentration (ng/µL)	Number	cobas® Factor II and Factor V Test Results		
		Number (%) Correct	Number (%) Incorrect	Number (%) Invalid
Undiluted*	120	120 (100%)	0 (0%)	0 (0%)
1	480	480 (100%)	0 (0%)	0 (0%)
0.3	480	480 (100%)	0 (0%)	0 (0%)
0.1	480	480 (100%)	0 (0%)	0 (0%)
0.03	480	480 (100%)	0 (0%)	0 (0%)
0.01	480	473 (98%)	0 (0%)	7 (2%)
0.003	480	86 (18%)	0 (0%)	394 (82%)
0.001	480	0 (0%)	0 (0%)	480 (100%)
0.0003	480	0 (0%)	0 (0%)	480 (100%)
0.0001	480	0 (0%)	0 (0%)	480 (100%)
0	480	0 (0%)	0 (0%)	480 (100%)

*6 to 38 ng/µL

4.2. Analytical sensitivity (Upper Limit)

To evaluate higher concentrations of DNA input for the **cobas®** Factor II and Factor V Test, genomic DNA was isolated from four K_2EDTA whole blood samples using three different commercial DNA isolation methods, and concentrated genomic DNAs from cell lines were added to yield total DNA concentrations of 300 ng/µL , 150 ng/µL and 75 ng/µL. Genomic DNAs from Factor II heterozygous, Factor V heterozygous and Factor V homozygous mutant cell lines were added to genomic DNAs from whole blood samples of the same Factor II and Factor V genotypes. Factor II homozygous mutant cell line DNA was added to genomic DNA from a leukocyte depleted whole blood (LDWB) sample. The genomic DNA samples at 300 ng/µL, 150 ng/µL and 75 ng/µL were tested with 24 replicates with each of two kit lots, for

a total of 48 replicates per concentration per genomic DNA sample. The genomic DNA samples from whole blood without added cell line DNA were tested with six replicates with each kit lot, for a total of 12 replicates per genomic DNA sample. All samples at 300 ng/µL, 150 ng/µL and 75 ng/µL yielded the correct Factor II and Factor V genotype results in all tests (Table 3).
The LDWB samples without added cell line DNA yielded Invalid results, as expected; the other genomic DNA samples from whole blood yielded correct Factor II and Factor V results.
The highest recommended DNA input concentration is 150 ng/µL.

Table 3: Testing of higher DNA input for the cobas® Factor II and Factor V Test

		cobas® Factor II and Factor V Test Results Number (%)		
Concentration (ng/µL)	**Number**	**Number (%) Correct**	**Number (%) Incorrect**	**Number (%) Invalid**
300	576	576 (100%)	0 (0%)	0 (0%)
150	576	576 (100%)	0 (0%)	0 (0%)
75	576	576 (100%)	0 (0%)	0 (0%)
Genomic DNA from neat whole blood samples (6 to 38 ng/µL)	108	108 (100%)	0 (0%)	0 (0%)
Leukocyte depleted whole blood sample*	36	36 (100%)	0 (0%)	0 (0%)

*Eluates from leukocyte-depleted whole blood sample yielded “Invalid” results due to the absence of leukocytes. These expected results are considered “correct” for the purpose of the study.

4.3. DNA Extraction Method Study

Genomic DNA was isolated from fifteen whole blood samples using three commercially available DNA isolation methods according to the manufacturer’s instructions, by two operators for three days, for a total of six DNA isolations per sample with each DNA isolation method. Each isolated genomic DNA sample was tested in triplicate with the **cobas®** Factor II and Factor V Test (Table 4). One hundred percent of the results with the **cobas®** Factor II and Factor V Test were in agreement with the Factor II and Factor V genotype by bi-directional Sanger sequencing. One genomic DNA sample isolated with method A was excluded from the results. It was rust colored and yielded invalid results in all three tests. Isolated DNA samples should appear clear and colorless. DNA samples with any appearance other than clear and colorless should not be tested as they may yield invalid or incorrect results.

Table 4: Results by DNA Isolation Methods

DNA Isolation Method	Total Number of		Number (%) of			
	DNA Isolations	Tests	Correct Results		Incorrect Results	Invalid Results
A	90	270	267[a] (98.9%)	(96.8 – 99.8)[b]	0 (0.0%)	3[a] (1.1%)
B	90	270	268[c] (99.3%)	(97.4 – 99.9)[b]	1[c] (0.4%)	1[c] (0.4%)
C	90	270	270 (100.0%)	(98.6 – 100)[b]	0 (0.0%)	0 (0.0%)
Total	270	810	805 (99.4%)	(98.6 – 99.8)[b]	1 (0.1%)	4 (0.5%)

[a] One of 90 DNA samples isolated with method A was rust-colored and generated 3 invalid results. Only clear and colorless DNA samples should be tested. DNA samples with any appearance other than clear and colorless should not be tested, as they may yield invalid or incorrect results.

[b] 95% two-sided, confidence interval

[c] The triplicate results from one sample isolated with method B were inconsistent: 1 correct, 1 incorrect, 1 invalid. The sample eluate was re-tested in triplicate and all results were correct upon re-testing.

4.4. Analytical Specificity (Potentially Interfering Mutation Study)

To determine the effect of known single nucleotide polymorphisms (SNPs) close to the Factor V Leiden mutation (1691G>A) and the Factor II (prothrombin) mutation (20210G>A), plasmid DNAs containing eight known SNPs (20207A>C, 20209C>T, 20218A>G, 20221C>T, 1689G>A, 1690C>T, 1692A>C and 1696A>G) were tested. The Factor II SNP plasmids and the Factor V SNP plasmids were wild type at positions 20210 and 1691, respectively. Each SNP plasmid DNA was tested alone, and in combination with wild type Factor II plasmid DNA, wild type Factor V plasmid DNA, wild type Factor II and wild type Factor V plasmid DNAs, and with genomic DNA from wild type whole blood.

None of the SNP plasmids caused false positive results for the Factor II (prothrombin) or Factor V Leiden mutations, and none of the SNP plasmids interfered with detection of the wild type Factor II or Factor V sequences. All four Factor II SNP plasmids and three of four Factor V SNP plasmids were detected as wild type Factor II or Factor V DNA, respectively. One Factor V SNP plasmid (1689G>A) was not detected by the **cobas®** Factor II and Factor V Test. If this SNP is present on both alleles, the test result would be Invalid.

4.5. Potential Interfering Substances Study

Triglycerides (37 mM), bilirubin (conjugated and unconjugated, 342 µM), and cholesterol (13 mM) did not interfere with the **cobas**® Factor II and Factor V Test when added to whole blood at the test concentrations recommended in CLSI guideline, EP07-A2.11 Hemoglobin did not interfere with the **cobas**® Factor II and Factor V Test when spiked into whole blood to yield a total hemoglobin concentration of ~25.8 - 31 g/dL.

The anticoagulant K_2EDTA did not interfere when it was tested at 5.7 mg/mL, which is approximately 3 times the concentration of K_2EDTA in whole blood when the blood collection tube is filled to capacity. Heparin, coumadin (Warfarin), rivaroxaban (Xarelto), and dabigatran etexilate (Pradaxa) did not interfere with the **cobas®** Factor II and Factor V Test.

A commercial extraction buffer containing guanidine hydrochloride, a common ingredient in commercial DNA extraction buffers, interfered with the **cobas®** Factor II and Factor V Test, causing invalid results when it was present in genomic DNA samples at a concentration of 2.5% (v/v). Ethanol, a common ingredient in the wash buffers of commercial DNA isolation methods, interfered with the **cobas®** Factor II and Factor V Test when added to genomic DNA samples to a concentration of 5% (v/v). For both substances, observed interference caused invalid results.

4.6. Lot-to-Lot Repeatability

The lot-to-lot repeatability of the **cobas®** Factor II and Factor V Test was evaluated by testing genomic DNA samples isolated from seven K_2EDTA whole blood samples with three lots of the **cobas®** Factor II and Factor V Test. The study was conducted over five non-consecutive days with two operators, two **cobas z** 480 analyzers, one run per day per kit lot, and two replicates per run, for a total of 60 replicates of each sample (Table 5). The **cobas®** Factor II and Factor V Test results were compared to the Factor II and Factor V genotype by bi-directional Sanger sequencing. The overall agreement between **cobas®** Factor II and Factor V Test results and nucleic acid sequencing was 100% across all samples and reagent lots (one-sided, lower 95% confidence limit 99.3%)

Table 5: Lot-to-lot Repeatability

Sample ID	Factor II Genotype	Factor V Genotype	Number of Tests per Lot	Number (%) Correct Genotype Results			
				Lot 1	Lot 2	Lot 3	All Lots
S1	Wild type	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S2	Wild type	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S3	Wild type	Heterozygous	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S4	Heterozygous	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S5	Wild type	Homozygous mutant	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S6	Homozygous mutant	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S7	Heterozygous	Heterozygous	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
Total			**140**	**140 (100%)**	**140 (100%)**	**140 (100%)**	**420 (100%)**

5. CLINICAL PERFORMANCE EVALUATION

5.1. Method Comparison Study

5.1.1. Study Objective

The objective of this investigational protocol was to compare the performance of the **cobas**® Factor II and Factor V Test to bi-directional Sanger sequencing for the identification of the Factor II and Factor V mutations in DNA isolated from whole blood specimens from patients with suspected thrombophilia.

5.1.2. Methodology

5.1.2.1. Study Design

A total of 300 specimens from patients whose routine medical care called for Factor II and/or Factor V measurements were obtained to represent the intended use population. The commercial vendors that provided these samples indicated that the collection included: individual Factor II and Factor V heterozygous mutations, individual homozygous Factor II and Factor V mutations, compound heterozygous mutations, and samples that were wild type for Factor II and Factor V Table 6.

There were 284 samples that were whole blood and represented frequently found genotypes for Factor II and Factor V. The majority of rare mutations such as Factor II homozygous/ Factor V Wild Type were acquired as DNA samples without identified extraction methodology.

Table 6: Factor II and Factor V genotypes included in the study

Factor II (F2)	Factor V (F5)	Specimen Type
Wild Type (WT F2)	Wild Type (WT F5)	Whole Blood
Wild Type (WT F2)	Heterozygous (HET F5)	Whole Blood
Heterozygous (HET F2)	Wild Type (WT F5)	Whole Blood
Heterozygous (HET F2)	Heterozygous (HET F5)	Whole Blood
Wild Type (WT F2)	Homozygous Mutant (MUT F5)	Whole Blood
Homozygous Mutant (MUT F2)	Wild Type (WT F5)	Whole Blood and DNA

5.1.2.2. Statistical Methods

The performance of the **cobas**® Factor II and Factor V Test was evaluated separately for Factor II and Factor V genotypes by using bi-directional Sanger sequencing as the reference method. Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and Overall Percentage Agreement (OPA) were estimated at the genotype level (Wild Type, Heterozygous, and Homozygous Mutant) separately for Factor II and Factor V.

5.1.3. Results

A total of 300 samples were tested for Factor II and Factor V by both **cobas**® Factor II and Factor V Test and Sanger sequencing. All runs and tests were valid for **cobas**® test and no repeat tests were performed. A test result was classified as correct for Factor II or Factor V, if both the **cobas**® test and Sanger sequencing detect the same genotype. A test result was classified as an incorrect for Factor II or Factor V if the **cobas**® test and Sanger sequencing detect a different genotype for Factor II or Factor V.

Table 7 presents agreement between the **cobas**® test and Sanger sequencing for Factor II results. The OPA between the two tests was 100% with a two-sided 95% lower confidence bound (exact method) of 98.78%. Both PPA and NPA were 100% with lower confidence bounds of

97.59% for PPA and 97.55 % for NPA. The percent agreement of correct results for Heterozygous and Homozygous Mutant genotypes were both 100%.

Table 7: Performance of the cobas® Factor II and Factor V Test using Bi-Directional Sanger Sequencing as a Reference for the Identification of Factor II Genotype

Genotype by Sequencing	Total Samples Tested	Correct Calls[1]	No calls or Invalid Results[2]	Missed or Incorrect Calls[3]	Percent Agreement	95% LCB[4]
Wild Type	149	149	0	0	100% **(NPA)**	97.55%
Positive	151	151	0	0	100% **(PPA)**	97.59%
Heterozygous	130	130	0	0	100%	97.20%
Mutant[5]	21	21	0	0	100%	83.89%
Total	300	300	0	0	100% **(OPA)**	98.78%

[1] **cobas**® test Factor II genotype results in agreement with the Factor II genotype by sequencing
[2] Invalid or failed **cobas**® result (no Factor II genotype call)
[3] **cobas**® test Factor II genotype results discordant with the Factor II genotype by sequencing
[4] Two-sided 95% lower confidence boundary is calculated using Exact method
[5] Homozygous mutant

Table 8 presents agreement between the **cobas**® test and Sanger sequencing for Factor V results. The OPA between the two tests was 100% with a two-sided 95% lower confidence bound (exact method) of 98.78 %. Both PPA and NPA were 100% with lower confidence bounds of 97.62% for PPA and 97.52 % for NPA. The percent agreement of correct results for Heterozygous and Homozygous Mutant genotypes were both 100%.

Table 8: Performance of the cobas® Factor II and Factor V Test using Bi-Directional Sanger Sequencing as a Reference for the Identification of Factor V Genotype

Genotype by Sequencing	Total Samples Tested	Correct Calls[1]	No calls or Invalid Results[2]	Missed or Incorrect Calls[3]	Percent Agreement	95% LCB[4]
Wild Type	147	147	0	0	100% **(NPA)**	97.52%
Positive	153	153	0	0	100% **(PPA)**	97.62%
Heterozygous	130	130	0	0	100%	97.20%
Mutant5	23	23	0	0	100%	85.18%
Total	300	300	0	0	100% **(OPA)**	98.78%

[1] **cobas**® test Factor V genotype results in agreement with the Factor V genotype by sequencing
[2] Invalid or failed **cobas**® result (no Factor V genotype call)
[3] **cobas**® test Factor V genotype results discordant with the Factor V genotype by sequencing
[4] Two-sided 95% lower confidence boundary is calculated using Exact method
[5] Homozygous mutant

Table 9: Performance of the cobas® Factor II and Factor V Test Using Bi-Directional Sanger Sequencing as a Reference for the Identification of Combined Factor II and Factor V Result

Bi-Directional Sanger sequencing Result	cobas® Factor II and Factor V Test Result						Total
	HET F2/ HET F5	HET F2/ WT F5	MUT F2/ WT F5	WT F2/ HET F5	WT F2/ MUT F5	WT F2/ WT F5	
HET F2/ HET F5	25	0	0	0	0	0	25
HET F2/ WT F5	0	105	0	0	0	0	105
MUT F2/ WT F5	0	0	21	0	0	0	21
WT F2/ HET F5	0	0	0	105	0	0	105
WT F2/ MUT F5	0	0	0	0	23	0	23
WT F2/ WT F5	0	0	0	0	0	21	21
Total	25	105	21	105	23	21	300

5.1.4. Conclusion

The results of this Method Comparison Study support the intended use of the **cobas**® Factor II and Factor V Test for diagnostic purposes as an aid in the clinical management of patients with suspected thrombophilia.

5.2. Clinical Reproducibility Study

5.2.1. Study Objective

The objective of this study was to evaluate the reproducibility of the **cobas**® Factor II and Factor V Test for the detection and genotyping of the Factor II (prothrombin) G20210A and Factor V Leiden (G1691A) mutations.

5.2.2. Methodology

5.2.2.1. Study Design

The reproducibility study included three manual whole blood sample preparation methods. These three whole blood DNA sample preparation kits were intended to be representative of commercially available sample preparation kits for DNA extraction from whole blood.

Reproducibility of the **cobas**® Factor II and Factor V Test was evaluated with K_2EDTA whole blood samples and genomic DNA samples across the following factors:

- **Sample Preparation Method:** One **(**1) sample preparation method per site
- **Reagent Lot:** Three (3) reagent lots, 1 lot per site
- **Test Site:** Three (3) sites (2 external and 1 internal)
- **Operator:** Two (2) operators per site
- **Instrument:** One (1) instrument per site
- **Run:** One (1) run per operator per day
- **Days:** Five (5) non-consecutive days per reagent lot

Reproducibility was assessed using a nine-member panel: four unique K_2EDTA blood samples, three contrived blood samples and two extracted genomic DNA (gDNA) samples diluted to 0.2 ng/µL.

Table 10: Description of Panel Members

Panel Member	Genotype of Panel Member		Sample Type
01	Factor II Wild Type	Factor V Wild Type	Whole Blood
02	Factor II Wild Type	Factor V Wild Type	Whole Blood
03	Factor II Wild Type	Factor V Heterozygous	Whole Blood
04	Factor II Heterozygous	Factor V Wild Type	Whole Blood
05	Factor II Wild Type	Factor V Homozygous mutant	Contrived Whole Blood
06	Factor II Homozygous mutant	Factor V Wild Type	Contrived Whole Blood
07	Factor II Heterozygous	Factor V Heterozygous	Contrived Whole Blood
08	Factor II Wild Type	Factor V Heterozygous	Genomic DNA
09	Factor II Heterozygous	Factor V Wild Type	Genomic DNA

5.2.2.2. Statistical Analyses

Data were summarized by the percentage of correct results, and the associated one-sided 95% exact lower confidence bounds (LCBs) or two-sided 95% exact confidence interval (CIs) and data were presented by panel member, genotype, site/sample preparation method and sample type (gDNA or whole blood).

For a valid test, a correct result was defined as agreement between the **cobas** test result and the panel member genotype as determined by Sanger sequencing; an incorrect result was defined as disagreement between the **cobas** test result and the panel member genotype as determined by Sanger sequencing. Test results are for both Factor II and Factor V and are either valid for both results or invalid for both results. A 'No Call' indicated that the test result was not available due to failed or invalid test result. Percent agreement of correct results between Sanger sequencing and **cobas**® Factor II and Factor V Test was determined from valid results.

5.2.3. Results

A total of 38 runs were performed in this study. A subset of 8 runs performed at site 1 with an expired sample preparation lot were determined as not acceptable and excluded from the statistical analysis. These runs were repeated with in-date reagents. The rest of 30 runs are all valid and meet all protocol requirements. A total of 540 tests were performed on the 9 panel members in the 30 valid runs, with 539 valid results and one invalid result. The following results are based on the dataset from these 30 runs.

5.2.3.1. Results for Factor II

Table 11 summarizes the overall agreement results of the reproducibility study for Factor II by overall genotype and testing site/sample preparation method. All results from valid tests were correct calls, leading to 100% agreement for each genotype. With the invalid result treated as an incorrect result, the percent agreement was 99.4% for HET and remained 100% for WT and MUT.

Table 11: Summary of Reproducibility Study -- Factor II

Genotype by Sequencing[a]	Correct Results/ Number of Samples Tested			Incorrect Results	Invalid Results[c]	Correct Results/ Number of Valid Results (%)	95% LCB[d]
Factor II	Site1[b]/ Lot1/ Method A	Site2[b]/ Lot2/ Method B	Site 3[b]/ Lot3/ Method C				
WT[e]	100/100	100/100	100/100	0	0	300/300 (100%)	98.78
HET[e]	60/60	59/60	60/60	0	1	179/179 (100%)	97.96
MUT[f]	20/20	20/20	20/20	0	0	60/60 (100%)	94.04

[a] WT: Wild Type; HET: Heterozygous; MUT: Homozygous Mutant

[b] Each site used different DNA isolation method (A, B, C) and different reagent lot

[c] Invalid result that was not retested

[d] Two-sided 95% Lower Confidence Bound (LCB) (exact method)

[e] At each site, 20 results were from gDNA samples, and 20 from contrived whole blood samples

[f] From contrived whole blood samples only

5.2.3.2. Results for Factor V

Table 12 summarizes the overall agreement results of the reproducibility study for Factor V by overall genotype and testing site/reagent lot/sample preparation method. All results from valid tests were correct calls, leading to 100% agreement for each genotype. With the invalid result treated as an incorrect result, the percent agreement was 99.4% for HET and remained 100% for WT and MUT.

Table 12: Summary of Reproducibility Study -- Factor V

Genotype by Sequencing[a]	Correct Results/ Number of Samples Tested			Incorrect Results	Invalid Results[c]	Correct Results/ Number of Valid Results (%)	95% LCB[d]
Factor V	Site1b/ Lot1/ Method A	Site 2b/ Lot2/ Method B	Site 3b/ Lot3/ Method C				
WT[e]	100/100	100/100	100/100	0	0	300/300 (100%)	98.78
HET[e]	60/60	59/60	60/60	0	1	179/179 (100%)	97.96
MUT[f]	20/20	20/20	20/20	0	0	60/60 (100%)	94.04

[a] WT: Wild Type; HET: Heterozygous; MUT: Homozygous Mutant

[b] Each site used different DNA isolation method (A, B, C) and different reagent lot

[c] Invalid result that was not retested

[d] Two-sided 95% Lower Confidence Bound (LCB) (exact method)

[e] At each site, 20 results were from gDNA samples, and 20 from contrived whole blood samples

[f] From contrived whole blood samples only

5.3. Conclusions

The submitted information in this 510(k) notification demonstrates that the **cobas**® Factor II and Factor V Test is as safe and effective as the predicate devices and therefore supports a Substantial Equivalence decision.