510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K172913

B. Purpose for Submission:

To obtain clearance for a new device

C. Measurand:

Factor II and Factor V

D. Type of Test:

Genotyping test

E. Applicant:

Roche Molecular Systems, Inc.

F. Proprietary and Established Names:

cobas® Factor II and Factor V Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 84.7280; Factor V Leiden DNA Mutation Detection Systems

2. Classification:

   Class II

3. Product code:

   NPR; Test, Factor II G20210A Mutations, Genomic DNA PCR, Factor V Leiden DNA Mutation Detection Systems

   NPQ; Test, Factor V Leiden Mutations, Genomics DNA PCR, Factor V Leiden DNA Mutation Detection Systems

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   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 K_2EDTA 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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   Prescription Use only

4. Special instrument requirements:

   cobas z 480 analyzer

I. 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.

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 real-time PCR thermal cycler (cobas z 480 analyzer) and a control unit (cobas 4800 System CU).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Factor II (Prothrombin) G20210A Kit
   Roche Factor V Leiden Kit

2. Predicate 510(k) number(s):

K033612
K033607 (DEN030005)

3. Comparison with predicate:

	Similarities		
Item	Device: cobas Factor II and Factor V Test (K172913)	Predicate: Roche Factor II (Prothrombin) G20210A Kit K033612	Predicate: Roche Factor V Leiden Kit 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 G1691A 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 except the predicate device was cleared on the LightCycler 1.2 analyzer.	Same except the predicate device was cleared on the LightCycler 1.2 analyzer.
Type of Test	Genotyping test	Same	Same
Target of Detection	Single nucleotide mutation	Same	Same
Indication for Use	Aid in the diagnosis of patients with suspected thrombophilia	Same	Same
Intended User	Healthcare professionals	Same	Same
Specimen Type	Purified DNA from	Same	Same

	Similarities		
Item	Device: cobas Factor II and Factor V Test (K172913)	Predicate: Roche Factor II (Prothrombin) G20210A Kit K033612	Predicate: Roche Factor V Leiden Kit K033607
	human blood samples		
	Differences		
Item	Device: cobas Factor II and Factor V Test (K172913)	Predicate: Roche Factor II (Prothrombin) G20210A Kit K033612	Predicate: Roche Factor V Leiden Kit K033607
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
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 z 480	Roche LightCycler version 1.2	Roche LightCycler version 1.2
Controls	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

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

CLSI EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline – 2nd Edition

L. Test Principle:

DNA is extracted offline from whole blood specimens collected in K_2EDTA 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. The amplification reactions generate a Factor II specific amplicon and 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. Depending upon the test order, results for one or both mutations are reported for each DNA sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

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. 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%). The table below summarizes the study results by individual lots and combined lots.

Sample ID	Factor II Genotype	Factor V Genotype	Number of Tests per Lot	Number (%) Correct Genotype Results			
				Lot 1	Lot 2	Lot 3	Lot 4
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%)

Clinical Reproducibility: A panel of nine specimens was tested at three sites, two operators per site, with one instrument per site and three lots of reagent lots with one lot per site. Each site had one operator which performed one run per day using one sample preparation method over five non-consecutive days. Reproducibility was assessed using a nine member panel as demonstrated in the table below: four K_2EDTA whole blood samples, three contrived whole blood samples and two extracted genomic (gDNA) samples diluted to 0.2 ng/µL. Below are tables which summarize the overall agreement results of the reproducibility study for Factor II and Factor V by overall genotype and testing site/sample preparation method.

Description of Specimens

Panel Number	Genotype of Specimen		Sample Type
1	Factor II Wild Type	Factor V Wild Type	Whole Blood
2	Factor II Wild Type	Factor V Wild Type	Whole Blood
3	Factor II Wild Type	Factor V Heterozygous	Whole Blood
4	Factor II Heterozygous	Factor V Wild Type	Whole Blood
5	Factor II Wild Type	Factor V Homozygous	Contrived Whole Blood
6	Factor II Homozygous Mutant	Factor V Wild Type	Contrived Whole Blood
7	Factor II Heterozygous	Factor V Heterozygous	Contrived Whole Blood
8	Factor II Wild Type	Factor V Heterozygous	Genomic DNA
9	Factor II Heterozygous	Factor V Wild Type	Genomic DNA

Factor II – Reproducibility Study Summary by Site

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]/Lot 1/Method A	Site 2[b]/Lot 2/Method B	Site 3[b]/Lot 3/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 a different DNA isolation method (A, B, C) and different reagent lot
[c]Invalid result 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 were from contrived whole blood samples
[f]From contrived whole blood samples only

Factor V – Reproducibility Study Summary by Site

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	Site1[b]/Lot 1/Method A	Site 2b/Lot 2/Method B	Site 3b/Lot 3/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 a different DNA isolation method (A, B, C) and different reagent lot
[c]Invalid result 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 were from contrived whole blood samples
[f]From contrived whole blood samples only

Factor II – Reproducibility Study Summary by Operator

Site	Operator	Genotype Panel[1]	Total Samples Tested	Correct Calls	Missed Calls	No Calls[2]	Percent Agrement % (Lower Bound of 95% CI[3])
Site 1	**Operator 1**	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
	Operator 2	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
Site 2	**Operator 1**	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
	Operator 2	WT	50	50	0	0	100.0 (92.89)
		HET	30	29	0	1	100.0 (88.06)
		MUT	10	10	0	0	100.0 (69.15)
Site 3	**Operator 1**	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
	Operator 2	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)

[1]WT: Wild Type; HET: Heterozygous; MUT: Homozygous Mutant
[2]Invalid results that were not retested
[3]95% CI = Two-sided 95% Exact Binomial Confidence Interval

Factor V – Reproducibility Study Summary by Operator

Site	Operator	Genotype Panel[1]	Total Samples Tested	Correct Calls	Missed Calls	No Calls[2]	Percent Agrement % (Lower Bound of 95% CI[3])
Site 1	**Operator 1**	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
	Operator 2	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
Site 2	**Operator 1**	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
	Operator 2	WT	50	50	0	0	100.0 (92.89)
		HET	30	29	0	1	100.0 (88.06)
		MUT	10	10	0	0	100.0 (69.15)
Site 3	**Operator 1**	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)
	Operator 2	WT	50	50	0	0	100.0 (92.89)
		HET	30	30	0	0	100.0 (88.43)
		MUT	10	10	0	0	100.0 (69.15)

[1]WT: Wild Type; HET: Heterozygous; MUT: Homozygous Mutant
[2]Invalid results that were not retested
[3]95% CI = Two-sided 95% Exact Binomial Confidence Interval

b. Linearity/assay reportable range:

Not applicable

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

Whole Blood Stability Study: This study was conducted to determine the stability of K_2EDTA whole blood specimens for isolation of gDNA for testing with the cobas Factor II and Factor V test. The study also compared the performance of the cobas Factor II and Factor V test on DNA isolated from freshly drawn whole blood samples to the performance on DNA isolated from whole blood samples frozen and thawed up to three times. DNA was isolated from K_2EDTA whole blood was collected from six donors and stored at -20°C and ≤ -70°C. In addition, one K_2EDTA blood collection tube from each donor was placed at: 2–8°C, 25°C and 32°C. The cobas Factor II and Factor V test yielded the correct genotype results for all samples, storage conditions and time intervals tested, including up to three freeze/thaw cycles at -20°C and ≤ -70°C. Genomic DNA for

testing with the cobas Factor II and Factor V test may be isolated from K_2EDTA whole blood specimens after storage at 2–8°C for up to seven days, at 9–30°C for up to three days. The performance of the cobas Factor II and Factor V test on whole blood samples frozen and thawed up to three times was comparable to the performance on fresh, never frozen samples.

Extracted Genomic DNA Stability Study: The stability of gDNA isolated from whole blood for testing with the cobas Factor II and Factor V test was determined by isolating gDNA from six K_2EDTA whole blood specimens using three commercially available genomic DNA isolation kits, and testing the gDNA samples with the cobas Factor II and Factor V test after storage at 2–8°C, -20°C and ≤ -70°C. All tests with the cobas Factor II and Factor V test for all samples, DNA isolation methods, storage conditions and time intervals yielded Factor II and Factor V genotype results in agreement with DNA sequencing. Genomic DNA isolated from K_2EDTA whole blood for testing with the cobas Factor II and Factor V test may be stored at 2–8°C for up to seven days or frozen at -20°C to below -70°C for at least nine weeks. Genomic DNA may be frozen and thawed up to three times.

Real-time and accelerated stability study: Three lots of the cobas Factor II and Factor V test kit were subjected to real-time stability testing at 2–8°C, and accelerated stability testing at 25°C and 37°C. The kit performance was assessed at each time point with the kit function release test and by testing genomic DNA extracted from four whole blood specimens at specified timepoints in the real-time study. The accelerated stability testing at 25°C and 37°C is complete. The real-time stability testing at 2–8°C is in progress, however real-time stability testing results through seven months from the date of manufacture is complete. The 25°C and 37°C accelerated stability studies on all three lots support a shelf life at 2–8°C of 18 months. The initial shelf life assignment for the cobas Factor II and Factor V test, based on the 25°C and 37°C accelerated stability studies is 15 months at 2–8°C. The 15-month shelf-life assignment will be confirmed and extended with real-time stability study data.

Open Reagent Stability Study: The stability of the cobas Factor II and Factor V test, after the reagents and controls have been used once, was determined by removing half the volume from multiple vials of the kit reagents and controls, storing and recapped vials at 2–8°C and testing them after approximately 31, 61 and 91 days of storage. At each time interval, the previously opened reagent and control vials were testing with the kit functional release test and by testing gDNA isolated from four K_2EDTA whole blood specimens. The study was conducted with one lot of the cobas Factor II and Factor V test. After opening and recapping, the reagents and controls were stable at 2–8°C for up to 90 days or the expiration date on the vials.

Activated Master Mix Stability Study: The stability of the activated Factor II and Factor V (F2F5) master mix (MMX) was determined by preparing activated F2F5 MMX, and testing the activated F2F5 MMX after storage at 32°C for 0, 1, 2, 2.5, 4 and 4.5 hours. Each time interval was tested with the kit functional test and by testing gDNA isolated from four K_2EDTA whole blood samples. The study was conducted with three lots of the cobas Factor II and Factor V test. Activated F2F5 MMX may be stored at room temperature for up to 4 hours.

d. Detection limit:

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 (see table below). 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. The table below summaries the study results.

Lower Limit Detection

Concentration (ng/µL)	Number	cobas Factor II and Factor V Test		
		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

Analytical Sensitivity - Upper Limit: To evaluate higher concentrations of DNA input for 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 DNA from cell lines were added to yield total DNA concentrations of 300 ng/µL, 150 ng/µL and 75 ng/µL. Genomic DNA from Factor II heterozygous, Factor V heterozygous and Factor V homozygous mutant cell lines were added to genomic DNA 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 (see table below). 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. The table below summarizes the study results.

Upper Limit Detection

		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 LDWB sample yielded "Invalid" results due to the absence of leukocytes. These expected results are considered "correct" for the purpose of this study.

e. Analytical specificity:

Substances: Six K_2EDTA whole blood samples representing Factor II and Factor V wild type and heterozygous genotypes were tested for potential interference by the following substances: triglycerides, bilirubin, cholesterol and hemoglobin, K_2EDTA, heparin, warfarin (Coumadin), rivaroxaban (Xarelto) and dabigatran etexilate (Pradaxa). 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. Hemoglobin did not interfere with the cobas Factor II and Factor V Test when added to whole blood at a concentration of 20 g/L. The anticoagulant K_2EDTA did not interfere when it was tested at 5.7 mg/mL, which is approximately three times the concentration of K_2EDTA in whole blood when the blood collection tube was filled to capacity. Heparin (3000 U/L), warfarin (32.5 µmol/L), rivaroxaban (1.5 µmol/L), and dabigatran etexilate (0.6 µmol/L) did not interfere with the cobas Factor II and Factor V Test. Correct genotype results were obtained for all replicates of all the gDNA samples.

For potential interference with extraction buffer and ethanol, these substances were added to gDNA isolated from K_2EDTA whole blood. 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.

Mutations: 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) in the probe binding regions of the cobas Factor II and Factor V Test 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.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

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 the genotypes as demonstrated in the table below.

Factor II and Factor V genotypes included in the method comparison study

Factor II (F2)	Factor V (F5)	Specimen Type
Wild Type (WT F2)	Wild Type (WT F5)	K_2EDTA whole blood
Wild Type (WT F2)	Heterozygous (HET F5)	K_2EDTA whole blood
Heterozygous (HET F2)	Wild Type (WT F5)	K_2EDTA whole blood
Heterozygous (HET F2)	Heterozygous (HET F5)	K_2EDTA whole blood
Wild Type (WT F2)	Homozygous (MUT F5)	K_2EDTA whole blood
Homozygous (MUT F2)	Wild Type (WT F5)	K_2EDTA whole blood and gDNA

One external site conducted testing with the cobas Factor II and Factor V test. One commercial laboratory performed bi-directional Sanger sequencing. The 300 samples were randomized and tested by two operators. All 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 the 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 detected the same genotype.

The table below presents agreement between the cobas test and Sanger sequencing for Factor II results. The Overall Percentage Agreement (OPA) between the two tests was 100% with a two-sided 95% lower confidence bound (exact method) of 98.78%. Both Positive Percent Agreement (PPA) and Negative Percent Agreement (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%.

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 Sanger 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

For Factor V, the table below 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%.

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%
Mutant[5]	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 Sanger 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

The table below presents method comparison study results for the combined Factor II and Factor V results.

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	25	105	21	105	23	21	300

b. Matrix comparison:

Matched frozen and fresh K_2EDTA whole blood samples were compared and resulted in 100% correct genotype results.

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):

DNA Extraction Method Study: Genomic DNA was isolated from 15 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 gDNA sample was tested in triplicate with the cobas Factor II and Factor V Test. 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 gDNA sample was excluded from the results as it was rust colored and yielded invalid results in all three tests. The table below summaries the results from the DNA extraction method study.

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%)	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 three invalid results. 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: one correct, one incorrect, one invalid. The sample eluate was re-tested in triplicate and all results were correct upon testing.

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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