

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

A. 510(k) Number:

k070597

B. Purpose for Submission:

New device

C. Measurand:

Factor II (Prothrombin)

Factor V Leiden

5, 10-Methylenetetrahydrofolate reductase (MTHFR)

D. Type of Test:

Genotype

E. Applicant:

Nanosphere INC

F. Proprietary and Established Names:

Verigene® F5 Nucleic Acid Test

Verigene® F2 Nucleic Acid Test

Verigene® MTHFR Nucleic Acid Test

G. Regulatory Information:

1. Regulation section:

21 CFR 864.7280 Factor V Leiden DNA mutation detection systems

2. Classification:

Class II

3. Product code:

NPR, Test, Factor II G20210A mutations, genomic DNA PCR

NPQ, Test, Factor V Leiden mutations, genomic DNA PCR

OMM, Test, 5, 10-Methylenetetrahydrofolate reductase mutations, genomic DNA PCR

NSU, Instrumentation for Clinical Multiplex Test Systems

4. Panel:

Hematology (81); Chemistry (75)

H. Intended Use:

1. Intended use(s):

The Verigene® F5 Nucleic Acid Test is an *in vitro* diagnostic for the detection and genotyping of a single point mutation (G to A at position 1691; also known as Factor V Leiden) of the human Factor V gene (F5; Coagulation Factor V gene) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene® System.

The Verigene® F2 Nucleic Acid Test is an *in vitro* diagnostic for the detection and genotyping of a single point mutation (G to A at position 20210 of the human Factor II gene (F2; prothrombin gene) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene® System.

The Verigene® MTHFR Nucleic Acid Test is an *in vitro* diagnostic for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5, 10-methylenetetrahydrofolate reductase gene (MTHFR) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene® System.

2. Indication(s) for use:
Same as Intended Use
3. Special conditions for use statement(s):
For Prescription Use Only.
4. Special instrument requirements:
Verigene® System

I. Device Description:

The Verigene® System consists of two instruments, the Verigene® Processor and the Verigene® Reader. It utilizes single-use, disposable test Cartridges to process and genotype multiple genes in a DNA sample in ~1 ½ hrs. Up to 8 Verigene® Processors may be connected to a single Verigene® Reader. Each processor contains 4 hybridization modules, and the modules can simultaneously run different tests.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Roche Diagnostic Corp. Factor V Leiden Kit
Roche Diagnostic Corp. Factor II (Prothrombin) G20210A Kit.
2. Predicate 510(k) number(s):
k033607, k033612
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	The Verigene® F5 Nucleic Acid Test is an <i>in vitro</i> diagnostic for the detection and genotyping of a single point mutation (G to A at position 1691; also known as Factor V Leiden) of the human Factor V gene (F5; Coagulation Factor V gene) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene® System.	The Factor V Leiden Kit is an <i>in vitro</i> diagnostic test for the detection and genotyping of a single point mutation (G to A at position 1691) of the human Factor V gene, from DNA isolated from human whole peripheral blood. The Factor V Leiden Kit is indicated as an aid to diagnosis in the evaluation of patients with suspected thrombophilia
Intended use	The Verigene® F2 Nucleic Acid Test is an <i>in vitro</i> diagnostic for the detection and genotyping of a single point mutation (G to A at position	The Factor II(Prothrombin) G20210OA Kit is an <i>in vitro</i> diagnostic test for the detection and genotyping

Similarities		
Item	Device	Predicate
	20210of the human Factor II gene (F2; prothrombin gene) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene® System.	of a single point mutations (G to A at position 202 10) of the human Factor II gene, from DNA isolated from human whole peripheral blood. The Factor II (Prothrombin) G20210A Kit is indicated as an aid to diagnosis in the evaluation of patients with suspected thrombophilia
Specimen Type	Peripheral whole blood	same

Differences		
Item	Device	Predicate
Intended use	The Verigene® MTHFR Nucleic Acid Test is an <i>in vitro</i> diagnostic for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5, 10-methylenetetrahydrofolate reductase gene (MTHFR) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene® System.	None
Detection method	Signal amplification Chemical amplification of reporter signal	DNA amplification (PCR)
Sample size	25 µL	10-20 µL in glass capillaries
Detection procedure	Single-image sensor where nanoparticles are illuminated using a fixed-wavelength light source	Optical detection of stimulated fluorescence
Detection Chemistry	SNP discrimination via oligonucleotide probes; detection via evanescent wave light scatter with nanoparticles	Paired hybridization probes using fluorescence resonance energy transfer (FRET)

Differences		
Item	Device	Predicate
Analysis time	1.5 hrs	Detection occurs at defined intervals during PCR cycle and can be viewed in real-time

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

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices

Class II Special Controls Guidance Document: Factor V Leiden DNA Mutation Detection Systems

CLSI EP5-A2; *Evaluation of Precision Performance of Quantitative Measurement Methods*

L. Test Principle:

The analysis sequence is the same for each of the three tests. After extracted and purified DNA, mixed with hybridization buffer, is loaded into the sample well of the test cartridge, it is ready for processing. An internal barcode reader reads the cartridge ID and sends the information to the Verigene® Reader. The reader establishes the hybridization parameters and starts the hybridization process.

The genotyping process occurs with a hybridization of the target analyze to a synthetic gene-specific oligonucleotide capture strand on the test cartridge's substrate. A synthetic mediator target-specific oligonucleotide is included with the test-specific sample buffer to form a hybridization "sandwich" with the gene sequence of interest. After washing, a probe composed of a gold nanoparticle with covalently bound oligonucleotide complementary to a sequence on the intermediate oligonucleotide, is introduced. A signal enhancement reagent is added to the hybridization chamber and reacts with the gold nanoparticle to amplify the signal for the Verigene® Reader scanning and analysis.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility testing included 4 studies, each using a different DNA samples extracted from whole blood. In the first study, each of the three test sites ran the same sample in duplicate.

Sites	F5	F2	MTHFR
1	HET	HET	HET
	HET	HET	HET
2	HET	HET	HET
	HET	HET	HET
3	HET	HET	HET
	HET	HET	HET

In the second study, one operator at Site 1 analyzed the same sample in duplicate each

day for three days.

Days	F2	F5	MTHFR
1	WT	HET	WT
	WT	HET	WT
2	WT	HET	WT
	WT	HET	WT
3	WT	HET	WT
	WT	HET	WT

In the third study, three operators at Site 2 each analyzed the same sample in duplicate.

Operators	F2	F5	MTHFR
1	WT	HET	HET
	WT	HET	HET
2	WT	HET	HET
	WT	HET	HET
3	WT	HET	HET
	WT	HET	HET

In the fourth study, one operator at Site 3 analyzed the same sample in duplicate using three lots of reagents.

Reagent Lots	F2	F5	MTHFR
1	HET	WT	HET
	HET	WT	HET
2	HET	WT	HET
	HET	WT	HET
3	HET	WT	HET
	HET	WT	HET

b. *Linearity/assay reportable range:*

NA

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

NA

d. *Detection limit:*

In 40 genomic samples diluted to 40ng/μL the total call rate for all three assays was 82%. There were no mis-calls.

e. *Analytical specificity:*

To simulate sample cross-contamination, a sample of known genotype was spiked with various amounts of a sample with a known but different genotype. The first sample consisted of a F2 (wild-type); F5 (mutant); MTHFR (wild type) and the

contaminating sample's genotype was: F2 (mutant); F5 (wild-type); MTHFR (hetero). The percent contaminating sample ranged from 0-50%. The results indicated that neither the call rate nor accuracy in a homozygous sample was affected in the presence of up to 10% contamination by volume of a sample with the opposite homozygous genotype. At least 20% contamination by volume of a sample with a heterozygous genotype is required to affect the call rate.

A study was performed to assess the interference of heparin, hemoglobin or magnetic beads in a purified DNA sample. Interference was observed in the 5X magnetic bead sample where the F5 signal was slightly lower than the control. The genotyping ration results showed slight variations but none led to an increase in no-calls or a mis-call.

f. Assay cut-off:

NA

2. Comparison studies:

a. *Method comparison with predicate device:*

Accuracy (percent agreement) was determined by comparison to bi-directional DNA sequencing:

A total of 287 samples, sixty-eight percent (68%) from patients undergoing “rule-out thrombophilia” testing, were analyzed at three sites using the Verigene® F5 / F2 / MTHFR Nucleic Acid Tests and by bi-directional sequencing analysis at an independent reference laboratory. All purified DNA samples were from whole blood collected using EDTA as the anticoagulant. Data comparison details for F5, F2, and MTHFR are shown in tables 3-5 below:

Table 3. Verigene® F5 Nucleic Acid Test method comparison results

Verigene® F5 Nucleic Acid Test				
Sequence analysis*		Wild-type (wt)	Heterozygous (het)	Mutant (mut)
	wt	253	0	0
	het	0	22	0
	mut	0	0	7

Table 4. Verigene® F2 Nucleic Acid Test method comparison results

Verigene® F2 Nucleic Acid Test				
Sequence analysis*		Wild-type (wt)	Heterozygous (het)	Mutant (mut)
	wt	258	0	0
	het	0	9	0
	mut	0	0	5

Table 5. Verigene® MTHFR Nucleic Acid Test method comparison results

Verigene® <i>MTHFR</i> Nucleic Acid Test				
Sequence analysis*		Wild-type (wt)	Heterozygous (het)	Mutant (mut)
	wt	125	0	0
	het	0	117	0
	mut	0	0	26

*Two DNA samples did not return complete bi-directional sequencing results and, therefore, were excluded from the data. Specifically, one sample did not produce results for any genotype reads (i.e., *F2*, *F5*, and *MTHFR*) and one sample returned no results for the *F2* genotype. These four genotype reads represent 0.7% of the total possible calls in the method comparison study. There were no samples excluded due to discrepancies between sequenced results.

The Verigene® *F5* / *F2* / *MTHFR* Nucleic Acid Tests demonstrated 100% Positive Percent Agreement (95%CI=98.9 to 100.0%) and 100% Negative Percent Agreement (95%CI=98.9 to 100.0%) in these studies based on data from calls made.

b. *Matrix comparison:*

NA

3. Clinical studies:

a. *Clinical Sensitivity:*

NA

b. *Clinical specificity:*

NA

c. *Other clinical supportive data (when a. and b. are not applicable):*

NA

4. Clinical cut-off:

NA

5. Expected values/Reference range:

NA

N. Instrument Name:

Verigene® System

O. System Descriptions:

1. Modes of Operation:

Closed system

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes **X** or No _____

3. Specimen Identification:

Barcode reader

4. Specimen Sampling and Handling:

DNA should be extracted using a DNA extraction method that provides DNA with the following characteristics:

Average purity: Optimal mean: 1.89 (typical range: 1.60 to 2.00 (A_{260} / A_{260}))

Concentration: 40 ng/μL to 400ng/μL
DNA elution: water or Tris-EDTA

5. Calibration:

The temperature control system on the Verigene® Processor device is the only component that requires calibration. This is performed at the time of manufacture or by a Nanosphere service technician.

6. Quality Control:

1. Automated on-line quality misprints that monitor instrument functionality, software performance, fluidics, test conditions, reagent integrity, and procedural steps in each assay each time a test is performed
2. Liquid materials

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the “Performance Characteristics” Section above:

Q. Proposed Labeling:

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

R. Conclusion:

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