510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k093974

B. Purpose for Submission:
New Device

C. Measurand:
Factor II (FII) (Prothrombin)
Factor V (FV) Leiden
5, 10 methylenetetrahydrofolate reductase (MTHFR)

D. Type of Test:
Qualitative genotyping test for single nucleotide polymorphism detection

E. Applicant:
Osmetech Molecular Diagnostics

F. Proprietary and Established Names:
eSensor® Thrombophilia Risk Test
eSensor® FII-FV Genotyping Test
eSensor® FII Genotyping Test
eSensor® FV Genotyping Test
eSensor® MTHFR Genotyping Test

G. Regulatory Information:

1. Regulation section:
   21 CFR §864.7280 Factor V Leiden DNA Mutation Detection Systems
   21 CFR §862.2570 Instrumentation for Clinical Multiplex Test Systems
2. Classification:
   Class II
3. Product code:
   NPQ: Test, Factor V Leiden Mutations, Genomic DNA PCR
   NPR: Test, Factor II G20210A 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):
   a. The eSensor® Thrombophilia Risk Test is an *in vitro* diagnostic for the detection and genotyping of Factor II (Prothrombin) G20210A, Factor V (Factor V Leiden) G1691A and MTHFR (human 5, 10 methylenetetrahydro-folate reductase gene) C677T and A1298C mutations in patients with suspected thrombophilia from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the eSensor® XT-8 System.
   b. The eSensor® FII-FV Genotyping Test is an *in vitro* diagnostic for detection

and genotyping of Factor II (Prothrombin) G20210A and Factor V (Factor V Leiden) G1691A mutations in patients with suspected thrombophilia from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the eSensor® XT-8 System.

c. The eSensor® FV Genotyping 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 (FV; 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 eSensor® XT-8 System.

d. The eSensor® FII Genotyping 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 (FII; 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 eSensor® XT-8 System.

e. The eSensor® MTHFR Genotyping Test is an *in vitro* diagnostic for the detection and genotyping of point mutations (C to T at position 677) and (A to C at position 1298) 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 eSensor® XT-8 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:
   eSensor® XT-8 System (k073720 and k090901)

I. Device Description:

The eSensor® Thrombophilia Risk Test consists of the following components:

- eSensor® Thrombophilia Risk Test Cartridge Pouch
- eSensor® TRT* PCR Mix
- Taq Polymerase
- Exonuclease
- Exonuclease Dilution Buffer
- eSensor® TRT* Signal Buffer
- XT Buffer-1
- XT Buffer-2
- eSensor® Thrombophilia Risk Test Product Insert

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Verigene® F5 Nucleic Acid Test
   Verigene® F2 Nucleic Acid Test
   Verigene® MTHFR Nucleic Acid Test
2. Predicate 510(k) number(s):
   k070597

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	FV Genotyping 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 (FV; Coagulation Factor V gene) in patients with suspected thrombophilia from isolated genomic DNA obtained from whole blood samples FII Genotyping 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 (FII; prothrombin gene) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples MTHFR Genotyping Test is an *in vitro* diagnostic for the detection and genotyping of a 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.	Same
Test Type	Qualitative genetic test for single nucleotide polymorphism detection	Same
Sample Type	Genomic DNA obtained from a human whole blood sample	Same
Target Of Detection	Single-nucleotide polymorphism	Same
DNA Extraction	Performed off-line	Same
Genes	Factor V Leiden, Factor II Prothrombin and MTHFR	Same
Genotyping Reaction Location	Test cartridge	Same

Similarities		
Item	Device	Predicate
Genotyping Principle	Sandwich hybridization test	Same
Assay Results	Assay signal results are interpreted by a software program and are assigned a result that is presented to the end-user in a report format	Same

Differences		
Item	Device	Predicate
Number Of Loci Genotyped	4 (FV, FII and MTHFR C677T and A1298C)	3 (FV, FII and MTHFR C677T)
Instrument Operating System	A single instrument with Processor and Reader.	Two instruments – the Verigene Processor and the Verigene Reader

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

Guidance for Industry and FDA Staff: Bundling Multiple Devices or Multiple Indications in a Single Submission.

Class II Special Controls Guidance Document: Factor V Leiden DNA Mutation Detection Systems - Guidance for Industry and FDA Staff.

L. Test Principle:

The eSensor® Thrombophilia Risk Test uses a solid-phase electrochemical method for determining the genotype of a defined panel of polymorphisms from purified genomic DNA isolated from human whole blood. In the process, the double-stranded PCR amplicons from the genomic DNA are digested with exonuclease lambda to generate single-stranded target DNA which is then mixed with a hybridization solution containing a pair of allele-specific oligonucleotide signal probes for each polymorphism. Each pair of signal probes is labeled with a genotype-specific ferrocene derivative.

The mixture of amplified target DNA sample and signal buffer is loaded onto a test cartridge containing single-stranded oligonucleotide capture probes that are covalently bound to gold-plated electrodes. Each target DNA binds to a capture probe. Each pair of working electrodes on the array contains a different capture probe.

The signal and capture probes are designed with sequences complementary to immediately adjacent regions on the corresponding target DNA sequence and so the signal probes bind to a complementary sequence on the target DNA. In this manner, a three-member complex is formed among capture probe, target, and signal probe based on sequence-specific hybridization. This process brings the 5'-end of the signal probe containing electrochemically active ferrocene labels into close proximity to the electrode surface. Upon completion of this process, the cartridge is inserted into the XT-8 Instrument.

In the XT-8 instrument, simultaneous hybridization of the three-member complex causes the ferrous ion in each ferrocene group to undergo cyclic oxidation and reduction, leading to loss or gain of an electron, and the generation of an alternating current at the electrode surface that is measured using voltammetry. Higher-order harmonic signal analysis also facilitates discrimination of ferrocene-dependent faradaic current from background capacitive current. Signals from the ferrocene labels are detected and measured by instrument software, and the ratio of signals allows identification of genotype. Genotyping boundaries and signal threshold for each polymorphism are pre-programmed into instrument software, and genotypes are called by comparison of the signal ratio observed for an unknown sample to the SNP-specific genotyping boundaries and signal threshold. Sequential analysis of each electrode allows genotyping of multiple mutations or polymorphisms.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Repeatability/Reproducibility: Two operators each from 3 different sites (2 external sites and 1 internal site) performed the testing in duplicate twice daily over 5 non-consecutive days using the same testing materials including gDNA from the following 5 blood samples:

Sample #	Sample Type	FII	FV	C677T	A1298C
1	gDNA from Blood	HET	WT	HET	WT
2	gDNA from Blood	WT	HET	MUT	WT
3	gDNA from Blood	WT	HET	WT	HET
4	gDNA from Blood	WT	WT	WT	MUT
5	gDNA from Blood	HET	MUT	WT	HET

There were 2/300 (0.67%) first pass no calls and zero miscalls. There was no statistical difference due to Site, Operator, Day or between replicates.

Site	Operator	Samples Tested	First pass			Final		% Agreement
			Correct Calls	No-Calls	Miscalls	Correct Calls	Miscalls	
Site A	1	50	50	0	0	50	0	100%
	2	50	50	0	0	50	0	100%
Site B	1	50	50	0	0	50	0	100%
	2	50	49	1	0	50	0	100%
Site C	1	50	49	1*	0	50	0	100%
	2	50	50	0	0	50	0	100%
All	All	300	298	2	0	300	0	100%
*This no-call was due to MTHFR-A1298C low signal. FII, FV and MTHFR-C677T were correctly called.								

Lot-to-Lot Reproducibility: A total of 5 genomic DNA samples, containing positive calls for FII, FV, and MTHFR C677T and A1298C, were tested in duplicate using three different kit lots of the eSensor® Thrombophilia Risk Test. A single operator executed the entire study. Results showed that all samples gave 100% agreement with DNA sequencing, and all genotypes were

correctly called after the first pass except a single first pass no-call result for FII (1/60), which was correctly called after repeat testing.

Genomic DNA Extraction Reproducibility: Three commonly used extraction methods were used to process the following 6 EDTA-blood samples:

Sample ID	Sample Type	FII	FV	C677T	A1298C
1	Whole Blood	WT	MUT	HET	WT
2	Whole Blood	HET	WT	WT	HET
3	Whole Blood	WT	HET	MUT	WT
4	Whole Blood	WT	WT	WT	MUT
5	Whole Blood	WT	WT	WT	MUT
6	Whole Blood	WT	WT	HET	HET

The 18 extracted samples was assayed in singlet in a single run using a single kit lot of the eSensor® Thrombophilia Risk Test. All samples, regardless of extraction methods, were correctly called on the first pass and gave 100% agreement with DNA sequencing.

b. *Linearity/assay reportable range:*
Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Real-Time Stability Study
The ongoing Kit Stability Study tests 5 gDNA samples representing all FII-FV-MTHFR mutations and genotypes in triplicate at each time point. The interim test results have demonstrated 3 month stability for the eSensor® Thrombophilia Test.
Opened Cartridge Pouch Stability Study
A WT gDNA sample was used to test once-opened pouches of eSensor® Thrombophilia Risk Test that had been stored at 30ºC and ambient humidity for 0, 33 and 61 days. The results showed that once-opened pouches of eSensor® Thrombophilia Risk Test are stable for 8 weeks (56 days) at 30ºC and ambient humidity.
Reagent Freeze-Thaw Stability Study
Aliquots of frozen materials included in the eSensor® Thrombosis Risk Genotyping Test Reagent Pack except Taq polymerase and Exonuclease kit components were subject to 7 freeze-thaw cycles prior to the final thaw at the time of testing. The genotyping testing was performed in quadruplet using four gDNA samples, which cover heterozygous genotypes for all mutations. Results showed that all samples were correctly called using reagents that underwent repeated freeze-thaw cycles or those that were thawed only once at the time of testing.
Sample Hybridization Stability In Cartridge Loading Reservoir
Samples with WT, MUT and HET genotypes were used to evaluate genotyping performance of the hybridization solution loaded on cartridges and stored at ambient temperature for 0 or 8.5 hrs. Results showed that hybridization solution loaded on the cartridges was stable for 8 hrs prior to running on the XT-8 instrument.

Double Stranded Amplicon Stability

Aliquots of a pooled sample of WT amplicons were stored at 4°C or -20°C for 0 or 9 days and tested in quintuplet for the yield of all 4 fragments of dsDNA. The results showed no amplicon stability failures in this study and supported the claim that dsDNA stored at 4°C or -20°C is stable for 7 days.

Single Stranded (Exonuclease Digested) Amplicon Stability

Aliquots of a pooled sample of exonuclease-digested WT amplicons were stored at 4°C or -20°C for 0 or 9 days and genotyped in quadruplet. The results showed that all samples were genotyped correctly and supported the claim that ssDNA stored at 4°C or -20°C is stable for 7 days.

d. *Detection limit:*

Two genomic DNA samples with different genotypes (one heterozygous for both FV and MTHFR A1298C, and the other heterozygous for both FII and MTHFR C677T) were extracted from whole blood stored in EDTA. They were serially diluted and tested 20 times each at five (5) different input amounts of 0.1, 1.0, 10, 100 and 500 ng. The limits of detection were determined to be 1.0 – 500 ng of input gDNA when all samples were called correctly for FII, MTHFR C677T and MTHFR A1298C.

e. *Analytical specificity:*

Probe Specificity

Individual amplicon targets (WT and MUT) for each mutation were generated by uniplex amplification of specific cell line gDNA samples and used to determine the overall specificity resulting from hybridization of signal probes and targets to their corresponding immobilized capture probes. Single stranded targets (at a concentration of 35nM in solution) from these amplicons were generated by exonuclease digestion prior to execution of the study.

No signals were observed on the blank target hybridized cartridges except for the expected signal on positive control pads, indicating the absence of cross-reactivity between signal probes and capture probes. Each target tested hybridized to the corresponding capture probe with the expected signal pattern.

Interfering Substances

Test performance was not affected by addition of the following substances to two whole blood samples of different genotypes prior to extraction:

- Heparin (3,000 U/L)
- Cholesterol (250 mg/dL)
- Bilurubin (30 mg /dL whole blood).
- Hemoglobin (~20g /dL whole blood).
- EDTA (at a concentration equivalent to 5-fold higher than that provided by a standard EDTA blood collection tube)

Interfering Mutations

The following interfering mutations were tested in 40 replicates alongside a Wild-Type control gDNA sample, and no effect was observed on multiplex amplification of target gene sequence, or genotyping by the eSensor® Thrombophilia Risk Test.

Non-Panel Mutation or Polymorphism	Panel Mutation
1692A>C 1689G>A 1696A>G	FV-1691G>A
20207A>C 20209C>T	FII-20210G>A

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison:

A total of 219 gDNA samples extracted from whole blood samples with A260-280 ratios of 1.0-2.9 were genotyped using the eSensor® Thrombophilia Risk Test and DNA sequencing. All samples gave 100% agreement with DNA sequencing.

Table: Comparison of Thrombophilia Risk Test and DNA Sequencing Results

FV Mutation														
Geno-type[1]	**First Pass Results**							**Final Results**						
	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB[2]	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB
WT	181	0	0	1	0	99.45	97.42%	182	0	0	1	0	100.00	98.37%
HET	0	27	0	1	0	96.43	84.15%	0	28	0	1	0	100.00	89.85%
MUT	0	0	9	0	0	100.00	71.69%	0	0	9	0	0	100.00	71.69%
FII Mutation														
Geno-type	**First Pass Results**							**Final Results**						
	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB
WT	183	0	0	1	0	99.46	97.45%	184	0	0	0	0	100.00	98.39%
HET	0	27	0	0	0	100.00	89.50%	0	27	0	0	0	100.00	89.50%
MUT	0	0	6	2	0	75.00	40.03%	0	0	8	0	0	100.00	68.77%
MTHFR (C677T) Mutation														
Geno-type	**First Pass Results**							**Final Results**						
	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB
WT	68	0	0	0	0	100.00	95.69%	68	0	0	0	0	100.00	95.69%
HET	0	118	0	2	0	98.33	94.85%	0	120	0	0	0	100.00	97.53%
MUT	0	0	31	0	0	100.00	90.79%	0	0	31	0	0	100.00	90.79%
MTHFR (A1298C) Mutation														
Geno-type	**First Pass Results**							**Final Results**						
	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB
WT	69	0	0	0	0	100.00	95.75%	69	0	0	0	0	100.00	95.75%
HET	0	117	0	3	0	97.50	93.67%	0	120	0	0	0	100.00	97.53%
MUT	0	0	30	0	0	100.00	90.50%	0	0	30	0	0	100.00	90.50%

Note:

[1]Genotype as determined with DNA sequencing;

[2]Lower boundary of the 95% confidence interval

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:
   The mutations being genotyped in the eSensor® Thrombophilia Risk Test are present at the following population frequencies in the general population:
   FV: 5%
   FII: 2%
   MTHFR-C677T: 40-50%
   MTHFR-A1298C: 40-50%

N. Instrument Name:
eSensor® XT-8 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:
   Manual labeling
4. Specimen Sampling and Handling:
   DNA should be extracted using a DNA extraction method that provides DNA with the following characteristics:
   Purity (A260/A280 ratio): 1.5 to 2.2;
   Concentration: 2 ng/µL – 100 ng/µL.
5. Calibration:
   No routine calibration or user maintenance is required.
6. Quality Control:
   Each test contains internal positive and negative controls to assure proper functioning of the system: Failure of either control will be indicated as "Invalid Control(s)" in the test results section of the report. The genotyping test results will not be reported for any sample for which a positive or negative control failure occurs.
   Positive Control: Each cartridge contains a capture probe that is complementary to a synthetic target DNA present in the hybridization mixture. The target also

contains ferrocene label and thus generates an appropriate signal in the assay. The positive control is designed to detect a systematic failure of the hybridization and/or detection processes. A lack of signal for the positive control indicates a genotyping assay failure. If a correct signal is observed for the positive control, but one or more genotyping assays are invalid due to low signals, then a failure of DNA isolation or PCR amplification or Exonuclease digestion is indicated.
Negative Control: A negative control is present on each cartridge, consisting of a capture probe that does not hybridize to any sequence within the target DNA or signal probes. Signals on the negative control indicate an assay system failure.
Hardware and Software Controls: The eSensor® XT-8 system contains additional controls in the hardware and software to enable proper performance as established under k073720 and k090901.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

1. Change of Device Name:
   In the original submission, the sponsor listed the candidate device as eSensorR FII-FV-MTHFR Genotyping Tests on XT-8 System. During the review process, the sponsor changed the device name to eSensor® Thrombophilia Risk Test on XT-8 System in response to FDA request for additional information, which did not contain any concerns or issues related to the device name.

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.