510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k093129

B. Purpose for Submission:
New Device

C. Measurand:
Factor II Prothrombin and Factor V Leiden genes in human blood specimens

D. Type of Test:
DNA Genotyping test

E. Applicant:
Illumina, Inc.

F. Proprietary and Established Names:
Illumina VeraCode® Genotyping Test for Factor V and Factor II

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
4. Panel:
   Hematology (81)

H. Intended Use:

1. Intended use(s):
   The VeraCode Genotyping Test for Factor V and Factor II is an in vitro diagnostic device for the detection and genotyping of Factor V Leiden G1691A and Factor II (Prothrombin) G20210A point mutations in DNA obtained from EDTA-anticoagulated human blood samples. The test is intended for use on the BeadXpress System. The VeraCode Genotyping Test for Factor V and Factor II on the BeadXpress System is indicated for use as an aid to diagnosis in the evaluation of patients with suspected thrombophilia.
2. Indication(s) for use:
   Same as Intended Use.
3. Special conditions for use statement(s):
   Prescription use only.
4. Special instrument requirements:
   BeadXpress® System (k093128), VeraScan software v. 2.0.17

I. Device Description:

The VeraCode Genotyping Test for Factor V and Factor II assay consists of reagents sufficient for 96 tests, consisting of two boxes containing pre-PCR and post-PCR reagents. The pre-PCR box contains the following reagents: MTR1 (1 x 1.2 mL), AB1 (1 x 4 mL), AOP1 (1 x 4.8 mL), ELM (1 x 4.8 mL), FSB (1 x 4.8mL), UB3 buffer (2 x 4.8 mL) and AE1 reagent (2 x 4.8 mL). The post-PCR box contains MSS

reagent (1 x 4.8 mL) and Fast Start Taq DNA Polymerase (1 x 60 µL), VW2 buffer (1 x 60 mL), a VeraCode FV/FII Bead Plate with holographically inscribed glass microbeads aliquoted in strip-well plates, a test-specific kit manifest file and sample sheet files (containing test specific outcome specifications and sample plate layout files used to interpret and report genotype results). A magnet plate is also required but sold separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   AutoGenomics INFINITI System for Factor II and Factor V
2. Predicate K number(s):
   k060564
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Illumina VeraCode® Genotyping Test for Factor V and Factor II	AutoGenomics INFINITI System for Factor II and Factor V
Intended Use/ Indications for Use	The VeraCode Genotyping Test for Factor V and Factor II is an in vitro diagnostic for the detection and genotyping of Factor V Leiden G1691A and Factor II (Prothrombin) G20210A point mutations in DNA obtained from EDTA anti-coagulated human blood samples. The test is intended for use on the BeadXpress System. The VeraCode Genotyping Test for Factor V and Factor II on the BeadXpress System is indicated for use as an aid to diagnosis in the evaluation of patients with suspected thrombophilia.	Same
Specimen type	Genomic DNA (gDNA) isolated from EDTA human whole blood	Same
Sample preparation	DNA extraction and purification performed off-line.	Same
Reference method	Bi-directional sequencing	Same
No. of mutations/ variants detected	2, Factor II (Prothrombin) G20210A and Factor V Leiden G1691A	Same
Assay result output	Assay results interpreted by a software program and assigned a genotype presented to the end user in a report format.	Same

Differences		
Item	Device	Predicate
Instrumentation	BeadXpress® System and VeraScan software v. 2.0.17	INFINITI System
Technology	Microbead array	Biofilm chip microarray
Detection Chemistry	Isolation of DNA from human blood sample, allele-specific primer extension and ligation, PCR amplification of the ligated primers, hybridization of the amplified product, and signal detection using fluorescence	Isolation of DNA from human blood sample, PCR amplification of the purified DNA, allele-specific primer extension, hybridization of the extended product, and signal detection using fluorescence
Input gDNA amount	2 µL (25 ng/µL)	5 µL (5–100 ng/µL)

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

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition

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

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

FDA Class II Special Controls Guidance, Factor V Leiden DNA Mutation Detection Systems

L. Test Principle:

The VeraCode Genotyping Test for Factor V and Factor II kit is an in vitro diagnostic device for mutation detection and genotyping of two mutations in two different genes related to increased risk for deep vein thrombosis, Factor V Leiden G1691A and Factor II (Prothrombin) G20210A point mutations in DNA obtained from EDTA-anticoagulated human blood. The VeraCode Genotyping Test for Factor V and Factor II utilizes polymerase chain reaction (PCR) primers, hybridization matrices, controls, holographically inscribed glass microbeads (VeraCode Microbead Plates) and assay-specific kit manifest files supplied with the reagent kit. The kit is run with processing reagents, general laboratory instrumentation and the Illumina BeadXpress System.

Genomic DNA is extracted from EDTA-anticoagulated whole blood. The assay uses allele-specific primer extension and ligation to detect sequence variants, followed by PCR amplification with fluorescent primers, hybridization to VeraCode beads, and collection of fluorescence data using the BeadXpress Reader.

The VeraCode Microbead Plates are assay specific, with each distinct holographic digitally coded microbead coated with a capture molecule. The BeadXpress® System is a fluidic microbead reader with VeraScan software for detection and genotyping multiple genes in a purified DNA sample. The BeadXpress includes a dual-color

laser detection system that enables optical scanning of multiplexed assays developed using the VeraCode digital microbead technology and VeraScan software.

The BeadXpress System includes red and green lasers for detecting and recording information from individual VeraCode assays (VeraCode Microbead Plates) loaded onto the BeadXpress Reader tray. After test samples are captured by the microbeads, fluorescently labeled reporter molecules complete the reaction. The BeadXpress Reader uses fluidics to collect and array the microbeads. The beads are scanned for their code and fluorescent signals, generating data as binary files, which are then used in downstream analysis with the assay-specific kit manifest files. The assay-specific kit manifest contains the parameters and cutoffs used to produce and report a genotype result.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A panel of 15 genomic DNA samples isolated from blood and consisting of 3 independent samples for each of the 5 possible genotypes (including Wild Type), were distributed to each of the three sites. At each site, two operators tested each sample in duplicate once a day for 5 non-consecutive days. Samples yielding invalid results were retested once and no incorrect calls were observed when the results were compared to bi-directional sequencing. Overall summary data based on the number of calls are shown below in Table 1 and 2. Summary data by operator and by site are shown in Tables 3 and 4.

Table 1. Summary of Reproducibility Study – Factor V

	No. of sample replicates			No. of FV Calls Before Repeat Testing						No. of FV Calls After Repeat Testing								
				Correct calls				%		# repeated[4]			Correct calls				%	
Genotype by sequencing	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	# No Calls[1]	Agree-ment	95% LCB[3]	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	No calls	Agree-ment	95% LCB
Wild Type	160	160	160	155	160	157	8	98.3	97.0	5	0	3	160	160	160	0	100	99.38
Heterozygous	80	80	80	79	78	80	3	98.8	96.8	1	2	0	80	79	80	1	99.58	98.04
Homozygous	60	60	60	58	58	60	4	97.8	97.8	2	2	0	60	60	60	0	100	98.35

Table 2. Summary of Reproducibility Study – Factor II

	No. of sample replicates			No. of FII Calls Before Repeat Testing						No. of FII Calls After Repeat Testing								
				Correct calls				%		# repeated[4]			Correct calls				%	
Genotype by sequencing	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	# No Calls[1]	Agree-ment	95% LCB[3]	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	No calls	Agree-ment	95% LCB
Wild Type	180	180	180	177	179	178	6	98.9	97.8	3	1	2	180	180	180	0	100	99.45
Heterozygous	60	60	60	59	60	60	1	99.4	97.4	1	0	0	60	60	60	0	100	98.35
Homozygous	60	60	60	57	59	59	5	97.2	94.3	3	1	1	60	60	60	0	100	98.35

[1] Sample failures or results generating a "no call" result and require repeating.

[2] Missed calls = wrong or incorrect calls

[3] One-sided 95% lower confidence bound

Table 3. Summary of Reproducibility Result by Operator – Factor V

	Site 1		Site 2		Site 3		% Agreement
	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2	
WT	100% (80/80)	100% (80/80)	100% (80/80)	100% (80/80)	100% (80/80)	100% (80/80)	100% (480/480)
HET	100% (40/40)	100% (40/40)	97.5% (39/40)	100% (40/40)	100% (40/40)	100% (40/40)	99.6% (239/240)
VAR	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (180/180)
No-Call* (1st pass)	4	4	4	0	2	1	15
No-Call after retest	0	0	1	0	0	0	1

* resolved upon retest

Table 4. Summary of Reproducibility Result by Operator – Factor II

	Site 1		Site 2		Site 3		% Agreement
	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2	
WT	100% (90/90)	100% (90/90)	100% (90/90)	100% (90/90)	100% (90/90)	100% (90/90)	100% (540/540)
HET	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (180/180)
VAR	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (180/180)
No-Call* (1st pass)	4	3	2	0	2	1	12

* resolved upon retest

Lot-to-Lot Reproducibility:

Three kit lots were tested by running five partial plates on a single BeadXpress Reader by a single operator over the course of five nonconsecutive days. Six gDNA samples from commercially available cultured cells were used to represent each possible genotype, including a compound heterozygous sample and each was represented 5 times (replicates) in each run, at or near 25 ng input. Two no template controls (NTCs) were also represented in each run. Genotypes were determined by the VeraScan software and Factor V & Factor II Kit manifest file. No-template controls (NTCs) were considered valid if signals for all functional loci were below threshold. An invalid NTC counts against Call Rate but not against Correct Call Rate. Call Rate and Correct Call Rate were calculated for each run and for each kit lot by pooling the data from the five runs. No sample failures resulting in an invalid result were noted for two of the three lots and one sample of the third lot generated an invalid (no call) result.

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

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The reference method is bi-directional sequencing.

Shelf life stability:
Summary stability information for an initial one month shelf life was provided, however real-time stability studies are still on-going.

Shipping stability:
Shipping stability was simulated by exposing three separate lots to cycling temperatures for specified durations from 22°C (4 hr.), 35°C (2 hr.), 30°C (36 hr.), and 35°C (22 hr.) to simulate 48 hour domestic shipping in the summer. Upon completion of temperature cycling, each kit was placed in dry ice and then functionally tested. Kits 1 and 3 each had one sample give an invalid result.

Carryover:
DNA samples were distributed across the reaction plate such that high concentrations of Factor V and Factor II heterozygote or homozygote variant samples were alternated with low concentrations of wild type or negative control samples in a checkerboard pattern. High concentrations of gDNA consisted of 500 ng input and low concentrations consisted of 25 ng input gDNA. All DNA samples genotyped correctly and there was no evidence of carryover from high concentration samples into low concentration samples. Three of the 24 NTC samples gave a signal over the acceptable threshold, however an investigation of the signals for each of the mutations showed that the only potential source for the contamination was several columns away with low concentration and other NTCs in between or were from wells after the affected wells which does not support potential carryover in two of the three cases. Three additional studies were performed but the original observations were not reproduced and the initial failures were not indicative system performance with respect to carryover.

d. *Detection limit:*
Analytical sensitivity for this test was defined as the lower limit of DNA input needed to generate a valid genotyping result. To establish a preliminary range of acceptable DNA input, a series of experiments was performed using Factor V and Factor II wild type, heterozygous and homozygous variant gDNA samples obtained from commercially available cell lines. DNA samples were suspended in TE buffer, such that the DNA input ranged from 1 – 1,000 ng per sample. Five replicates of each sample were run per plate and DNA input levels of 5 ng, 10 ng, 25 ng, 500 ng, and 1000 ng met the preliminary passing criteria where all replicates of all genotypes produce the correct result when compared to bi-directional sequencing. Twenty-five (25) and 500 ng were selected as the preliminary DNA input claim based on having a lower (10 ng) and a higher (1000 ng) input, respectively, that passed the preliminary passing criteria. To allow for sample failures unrelated to DNA input, one invalid

sample per run was allowed and one sample with a failed hybridization control was allowed. A preliminary range for a DNA input claim was established with a "low" and a "high" DNA input. The DNA input limits of 25 ng (5 ng/µL) and 500 ng (100 ng/µL) were tested with another two reagent kit lots and one additional VeraCode Bead Plate Factor V/Factor II lot and all replicates were successfully genotyped.

e. Analytical specificity:

To determine the potential effect of heparin on test results as interference may be observed from patients on heparin therapy, a concentrated solution of sodium heparin was spiked directly into prospectively drawn blood samples, as recommended in CLSI EP7-A2, at 100 U/dL, 300 U/dL, and 900 U/dL. High levels of heparin added to whole blood did not adversely affect assay performance on the resulting extracted DNA.

To rule out any potential interference by endogenous interfering substances (i.e., bilirubin, hemoglobin, and cholesterol) each was spiked into aliquots from two EDTA-anticoagulated blood samples (1 FV HET and 1 FII HET) to a final concentration, at or above those recommended in Appendix D of CLSI EP7-A2, at 684 µmol/L (bilirubin), 13 mmol/L (cholesterol), and 2 g/L (hemoglobin). Each spiked aliquot was tested with the VeraCode Bead Plate Factor V/Factor II and compared to an aliquot spiked with the individual carrier used to dilute the interferent. All samples genotyped equivalently across the entire experiment and no assay failures were observed in the external positive control samples or the samples extracted from blood spiked with potential interfering substances or vehicle only. Therefore elevated levels (as defined by CLSI guideline EP7-A2) of bilirubin, cholesterol, hemoglobin, and heparin in blood do not adversely influence the performance of the VeraCode Genotyping Test for Factor V and Factor II. The claimed genotypes for these DNA samples were confirmed by bidirectional sequencing.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Accuracy was measured as overall percent agreement of the genotyping results produced by the VeraCode Genotyping Test for Factor V and Factor II as compared to those resulting from bi-directional DNA sequencing. Ninety-two patient samples were accrued at each of the three sites, yielding a total of 276 patient samples, from pre-selected or unscreened patients undergoing Factor V testing and each site was provided with two archived DNA samples to serve as positive controls. The samples were then analyzed using the VeraCode and compared to bi-directional sequencing analysis performed at an independent reference laboratory. All genomic DNA samples were extracted from EDTA anti-coagulated whole blood samples. A summary of the make up of samples by site and summary results are shown in Tables 5-7 below.

Table 5. Summary of samples genotypes by site.

	Site 1	Site 2	Site 3
WT (for FV)	72	78	63
WT (for FII)	83	85	77
FVL Heterozygous	17	11	21
FVL Homozygous	3	3	8
FII Heterozygous	7	5	12
FII Homozygous	2	2	3

Table 6. Summary Method Comparison – Factor V

		Number of FVL Calls Before Repeat Testing					Number of FVL Calls After Repeat Testing					
Genotype by sequencing	# samples	Correct calls	# No Calls[1]	Missed Calls[2]	% Agreement	95% LCB[3]	Correct calls	# No Calls[1]	Missed Calls[2]	# Repeated samples[4]	% Agreement	95% LCB[3]
Wild Type	213	213	0	0	100	89.6	213	0	0	0	100	98.6
Heterozygous	49	46	3	0	93.88	84.93	49	0	0	3	100	94.07
Homozygous	14	13	1	0	92.86	70.33	13	1	0	1	92.86	70.33

Table 7. Summary Method Comparison – Factor II.

		Number of FII Calls Before Repeat Testing					Number of FII Calls After Repeat Testing					
Genotype by sequencing	# samples	Correct calls	# No Calls[1]	Missed Calls[2]	% Agreement	95% LCB[3]	Correct calls	# No Calls[1]	Missed Calls[2]	# Repeated samples[4]	% Agreement	95% LCB[3]
Wild Type	245	244	1	0	99.59	98.08	244	1	0	1	99.59	98.08
Heterozygous	24	23	1	0	95.83	81.71	24	0	0	1	100	88.27
Homozygous	7	7	0	0	100	65.18	7	0	0	0	100	65.18

[1] Sample failures or results generating a "no call" result and require repeating.
[2] Missed calls = wrong or incorrect calls
[3] One-sided 95% lower confidence bound

b. Matrix comparison:
Not applicable. Both assays use gDNA purified from EDTA anti-coagulated whole blood

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:
   Not applicable. Factor II (G20210A) and Factor V Leiden (G1691A) mutations are present in 2% and 5% of the general population, respectively.

N. Proposed Labeling:

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

O. Conclusion:

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