

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

A. 510(k) Number:

k100943

B. Purpose for Submission:

New device

C. Measurand:

Factor II

D. Type of Test:

Qualitative genotyping test for single nucleotide polymorphism detection

E. Applicant:

Hologic Inc.

F. Proprietary and Established Names:

Invader[®] 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
4. Panel:
81 Hematology

H. Intended Use:

1. Intended use(s):
The Invader[®] Factor II test is an *in vitro* diagnostic test intended for the detection and genotyping of a single point mutation (G to A at position 20210) of the human Factor II gene in isolated genomic DNA obtained from whole blood potassium EDTA samples from patients with suspected thrombophilia.
2. Indication(s) for use:
Same as Intended use
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
Multi-well fluorometer capable of:

Multi-Labeling Measurement Parameters	Measurement 1	Measurement 2
Read mode	Top	Top
Excitation wavelength	485/20 nm	560/20 nm
Emission wavelength	535/25 nm	612/10 nm
Gain	Manual	Manual
Number of flashes	10	10
Integration time	20 μ s	20 μ s

I. Device Description:

The Invader[®] Factor II test consists of the following components:

- Factor II Oligo Mix
- Universal Buffer
- Universal Enzyme Mix
- No DNA Control
- Factor II Wild Type Control
- Factor II Heterozygous Control
- Factor II Mutant Control
- Invader[®] Call Reporter™ Software – Version 5.3
- Invader[®] Factor II Software – Version 2.0

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
Roche Factor II (Prothrombin) G20210A Kit (k033612)
2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	An <i>in vitro</i> diagnostic test intended for the detection and genotyping of a single point mutation (G to A at position 20210) of the human Factor II gene in isolated genomic DNA obtained from whole blood potassium EDTA samples from patients with suspected thrombophilia.	Same
Target Population	Patients with suspected thrombophilia	Same
Specimen Type	Purified DNA isolated from human whole peripheral blood	Same
Target Amplification Technology	PCR	Same
Signal Generation Technology	Fluorescence Resonance Energy Transfer (FRET)	Same
Output Data	Assay signal results are interpreted by a software program and are assigned a genotype that is presented to the end user in a report format	Same

Differences		
Item	Device	Predicate
Allele Discrimination	Targeted cleavage of distinct FRET cassette bound to allele-	Melting curve analysis of hybridizations to allele-

Differences		
Item	Device	Predicate
	specific primary probe with a unique 5'-flap	specific FRET probes
Reaction Conditions	20-μL reaction in multiple plastic microtiter wells	15 μL reaction in glass capillaries loaded in a sample carousel
Signal Detection	End-point detection of amplified sequences after PCR amplification	Real-time detection of amplified sequences during PCR cycles
Hardware	Non-specified, third-party fluorometer and thermal cycler	LightCycler® Instrument V1.2 including a cycler and a fluorimeter
Software Interface	Java-based software installed on a standalone PC capable of converting raw fluorescence data into genotype calls	LightCycler® Software V3.5

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

- Guidance for Industry and FDA Staff – Class II Special Controls Guidance Document: Factor V Leiden DNA Mutation Detection Systems issued on March 16, 2004
- Guidance for Industry and FDA Staff - Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices issued May 11, 2005
- Guidance for Industry and FDA Staff – Format for Traditional and Abbreviated 510(k)s issued on August 12, 2005

L. Test Principle:

The Invader® Factor II test combines target amplification by PCR using third-party thermal cycler with signal generation by the Invader Plus® chemistry and signal detection using interpretative software and third-party fluorometer for genotyping of a single point mutation (G to A at position 20210) of the human Factor II gene.

During the signal generation phase, a discriminatory Primary Probe transiently hybridizes to the amplified target sequence along with an Invader® oligonucleotide, to form an overlapping structure. The 5'-end of the Primary Probe includes a 5'-flap that does not hybridize to the target DNA. The 3'-nucleotide of the bound Invader® oligonucleotide overlaps the Primary Probe, and does not hybridize to the target DNA. The Cleavase® enzyme recognizes this overlapping structure and cleaves off the unpaired 5'-flap of the Primary Probe, releasing it as a target-specific product. The Primary Probe is designed to have a melting temperature aligned with the Invader® reaction temperature so that under the isothermal reaction conditions (~63°C) the Primary Probes cycle on and off the target DNA. This allows for multiple rounds of Primary Probe cleavage for each DNA target resulting in an accumulation of released 5'-flaps. The released 5'-flap transiently hybridizes with a corresponding fluorescent resonance energy transfer (FRET) cassette forming an overlapping structure that is recognized and the fluorophore is cleaved from the FRET cassette by the Cleavase® enzyme. The 5'-flap is designed to have a melting temperature aligned with the Invader® reaction temperature, so that the 5'-flaps cycle on and off of the corresponding FRET cassettes. This allows for multiple rounds of FRET cassette cleavage for each 5'-flap, and an

accumulation of released fluorophore. When the FRET cassette is cleaved, a fluorophore and quencher are separated, generating detectable fluorescence signal.

The Invader[®] Factor II test uses two different discriminatory Primary Probes, one for the mutant allele and one for the wild type allele. Each Primary Probe is assigned a unique 5'-flap, and distinct FRET cassette, with a spectrally distinct fluorophore. The released 5'-flaps will bind only to their respective FRET cassettes to generate a target-specific signal, linking the wild type allele with one fluorophore (Fluorescence 1: RED) and the mutant allele with the second fluorophore (Fluorescence 2: FAM).

The Invader[®] Factor II software, in combination with Invader[®] Call Reporter™ software, is a data analysis software that provides a working template for the setup of reaction mixes and sample placement, and following the import of fluorescence data, it determines results and validity for controls and samples.

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 a panel of nine (9) whole blood samples, representing all possible genotypes including three (3) wild types (WT), three (3) heterozygous mutants (HET) and three (3) homozygous mutants (MUT).

There were 36/540 (6.7%) first pass No Calls due to “Invalid Control” result on two (2) independent runs. Upon retraining of the operator and retesting of the runs, all controls reported “Valid” and 34 samples were found to be in agreement with sequencing. Two (2) samples with Final No Call were called correctly upon re-extraction and re-testing. There were zero Miscalls, and there was no statistical difference due to Site, Operator or Day.

Site	Operator	Samples Tested	First pass			Final		% Agreement
			Correct Calls	No-Calls	Miscalls	Correct Calls	Miscalls	
Site 001	1	90	90	0	0	90	0	100%
	2	90	90	0	0	90	0	100%
Site 002	1	90	90	0	0	90	0	100%
	2	90	90	0	0	90	0	100%
Site 003	1	90	90	0	0	90	0	100%
	2	90	54	36	0	88	0	97.78%
All	All	540	504	36	0	538	0	99.63%

Lot-to-Lot Reproducibility: A total of four (4) genomic DNA samples (three (3) wild type and one (1) heterozygous) were tested in quadruplicate using three (3) different kit lots of the Invader[®] Factor II test. The percent agreement between Invader[®] Factor II test and sequencing was 100% (n=48).

Genomic DNA Extraction Reproducibility: Four commonly used extraction methods were used to process thirty (30) human whole blood samples and ten (10) leukocyte depleted whole blood (LDWB) spiked with cell lines with the following genotypes:

Sample Type	Number of Samples			
	WT	MUT	HET	Total
Human whole blood samples	20	0	10	30
LDWB spiked with cell lines	5	5	0	10
Total	25	5	10	40

The 160 extracted samples were assayed in singlet in a single run using a single kit lot of the Invader[®] Factor II test. With the exception of one sample that was removed from the study due to loss of traceability of the sample identification, all other 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):*

Quality Control:

Each test contains positive and negative controls to assure proper functioning of the system: Failure of any controls will be indicated as “Invalid” in the test results section of the report. The genotyping test result will not be reported for any sample for which a positive or negative control failure occurs.

Positive Control: The genotype controls (WT, HET, MUT) ensure reagents were assembled correctly and perform according to the specifications.

Negative Control: The No DNA Control is used by the interpretive software to set the “noise” component of the run for “signal-to-noise” calculations.

Hardware and Software Controls: The genotyping test result will not be reported for any sample for which a positive or negative control failure occurs.

Real-Time Stability Study

The ongoing Stability Study tests seven (7) gDNA samples including three (3) WT, two (2) HET and two (2) MUT genotypes using three (3) lots of Invader[®] Factor II product stored under two recommended conditions: 1) -20°C (Standard Storage of intermediate components) and 2) 4°-8°C (Standard Storage of Genotype-Specific Controls). Functional testing is performed in quadruplicate at each time point and the interim test results have demonstrated 7 months stability for the device.

Sample/Control	Genotype	T0 Result			T4 Result			T7 Result		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
Control 1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
Control 2	HET	HET	HET	HET	HET	HET	HET	HET	HET	HET
Control 3	MUT	MUT	MUT	MUT	MUT	MUT	MUT	MUT	MUT	MUT
gDNA 1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
gDNA 2	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
gDNA 3	HET	HET	HET	HET	HET	HET	HET	HET	HET	HET
gDNA 4	MUT	MUT	MUT	MUT	MUT	MUT	MUT	MUT	MUT	MUT
Percent Agreement		100	100	100	100	100	100	100	100	100

Reagent Freeze-Thaw Stability Study

Product in the final configuration was subject to 15 freeze-thaw cycles prior to the

final thaw at the time of testing. Functional testing was performed in replicate of 3-8 using genomic DNA samples isolated from cell lines, representing all possible genotypes including two (2) WT, two (2) HET and two (2) MUT. Results showed that all samples were correctly called using Invader[®] Factor II test reagents that underwent repeated freeze-thaw cycles.

d. *Detection limit:*

Two (2) genomic DNA samples with different genotypes (i.e., one WT and the other HET mutant) were extracted from whole blood collected in potassium EDTA. They were serially diluted and tested 40 times each at eight (8) different input amounts of (0.5, 5, 20, 40, 80, 200, 400, 800 ng/μL). The lower and upper limits of input gDNA when all samples were called correctly were determined to be 5 ng/μL and 80 ng/μL, respectively.

e. *Analytical specificity:*

Interfering Substances

Test performance was not affected by addition of the following substances to four (4) whole blood samples of different genotype (3 WT, 1 HET) prior to extraction:

- Heparin (1500 U/dL human whole blood)
- Cholesterol (300 mg/dL human whole blood)
- Bilirubin (10 mg/dL human whole blood)
- Hemoglobin (up to 0.2% in whole blood)
- Potassium EDTA (1.8 mg/mL human whole blood)
- Ethanol-based Wash Buffer (5% in DNA sample)

Interfering Mutations

The effect of interfering mutations on G20210A genotyping by the Invader[®] Factor II Test was evaluated with six (6) contrived DNA samples, including one (1) WT sample with no secondary polymorphism, one (1) HET sample and four (4) WT samples each with a known secondary polymorphism (i.e., A20207C, C20209T, A20218G, or C20221T). Forty replicates for each of the six (6) different samples were tested, and no effect of secondary polymorphisms was observed on G20210A genotyping by the Invader[®] Factor II Test.

Non-Panel Mutation or Polymorphism	Panel Mutation
20207A>C 20209C>T 20218A>G 20221C>T	20210G>A

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison:*

A total of 336 gDNA samples extracted from whole blood samples were genotyped using the Invader[®] Factor II Test and DNA sequencing. All samples gave 100% agreement with DNA sequencing.

Table: Comparison of Invader[®] Factor II Test and DNA Sequencing Results

Geno-	First Pass Results	Final Results
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type ^[1]	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB ^[2]	WT	HET	MUT	No-Call	Mis-Call	% Agree-ment	95% LCB
WT	305	0	0	0	0	100.00	99.02	305	0	0	0	0	100.00	99.02
HET	0	24	0	0	0	100.00	88.27	0	24	0	0	0	100.00	88.27
MUT	0	0	7	0	0	100.00	65.18	0	0	7	0	0	100.00	65.18

Note:

^[1]Genotype as determined with DNA sequencing;

^[2]Lower boundary of the 95% confidence interval

b. Matrix comparison:

Not applicable

c. Instrument Equivalency:

Twenty-nine (29) human whole blood samples and ten (10) leukocyte depleted whole blood samples spiked with cell lines were extracted using two (2) commonly used extraction methods. The extracts were tested with the Invader[®] Factor II test using three (3) commercially available thermal cyclers and the raw fluorescent data acquired on three (3) commercially available fluorometers. Results from the three (3) fluorometers were transferred into the interpretive software and genotype calls compared to bi-directional sequencing.

Concordance by Instrument			
Fluorometer	Thermal Cycler		
	1	2	3
A	78 of 78 = 100%	77 of 78 = 98.7%	78 of 78 = 100%
B	78 of 78 = 100%	77 of 78 = 98.7%	78 of 78 = 100%
C	78 of 78 = 100%	77 of 78 = 98.7%	78 of 78 = 100%

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 Factor II mutation being genotyped in the Invader[®] Factor II Test is present at a frequency of 1-2% in the general population.

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.