

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

**A. 510(k) Number:**

k082118

**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:**

Cepheid

**F. Proprietary and Established Names:**

Xpert™ HemosIL® FII & FV

**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:

NPR; Test, Factor II G20210A Mutations, Genomic DNA PCR, Factor V Leiden DNA mutation detection systems

NPQ; Test, Factor V Leiden Mutations, Genomic DNA PCR, Factor V Leiden DNA mutation detection systems

OOI; Real-time nucleic acid amplification systems

4. Panel:

81 Hematology, 75 Chemistry

**H. Intended Use:**

1. Intended use(s):

The Xpert™ HemosIL® Factor II & Factor V Assay is a qualitative *in vitro* diagnostic genotyping test for the detection of Factor II and Factor V alleles from sodium citrate or EDTA anticoagulated whole blood. The assay is performed on the Cepheid GeneXpert® Dx System. This test is intended to provide results for Factor II (G20210A) and Factor V Leiden (G1691A) mutations as an aid in the diagnosis in individuals 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:

Cepheid GeneXpert® Dx System (k060540)

**I. Device Description:**

The GeneXpert Dx System automates and integrates sample purification, nucleic acid amplification and detection of the target sequence in whole blood using real-time

PCR assays. The system consists of an instrument, personal computer, handheld barcode scanner, and pre-loaded software for running tests and viewing results. The system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated.

The Xpert HemosIL Factor II & Factor V Assay includes primers and probes for the detection of Factor II allele (at position 20210) and the Factor V gene (at position 1691) in sodium citrate or EDTA anticoagulated whole blood. Each assay kit consists of two single-use reagents (Reagent 1 and Reagent 2), one each of the Xpert HemosIL Factor II & Factor V disposable fluidic cartridge. Each cartridge contains a Probe Check Control (PCC) that verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability. The primer and probes in the Xpert HemosIL Factor II & Factor V Assay determine the genotype of the Factor II gene.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Roche Factor V Leiden Kit & Roche Factor II (Prothrombin) G20210A Kit
2. Predicate K number(s):  
k033607 & k033612
3. Comparison with predicate:

<b>Similarities</b>			
Item	Device	Predicate	
	Xpert HemosIL Factor II & Factor V Assay	Roche Factor II (Prothrombin G20210A (k033612))	Roche Factor V Leiden Kit (k033607)
Intended Use	Qualitative in vitro diagnostic genotyping test for the detection of Factor II and Factor V alleles from sodium citrate and EDTA anticoagulated whole blood	Same except detection of Factor II only in EDTA anticoagulated blood only	Same except detection of Factor V only in EDTA anticoagulated blood only
Indication for Use	Aid in the diagnosis in individuals with suspected thrombophilia	Same	Same
Technological Detection Principles	Amplification and detection system for nucleic acids using fluorescence detection	Same	Same

<b>Differences</b>			
Item	Device	Predicate	
	Xpert HemosIL Factor II & Factor V Assay	Roche Factor II (Prothrombin G20210A (k033612))	Roche Factor V Leiden Kit (k033607)

<b>Differences</b>			
<b>Item</b>	<b>Device</b>	<b>Predicate</b>	
Specimen Type	Sodium citrate and EDTA human whole blood	Purified DNA from EDTA human blood samples	Purified DNA from EDTA human blood samples
Sample Preparation	Automated On-line	Performed off-line	Performed off-line
Test Cartridge	Disposable single-use, multi-chambered fluidic cartridge	Disposable single-use PCR capillary	Disposable single-use PCR capillary
Instrument System	Cepheid GeneXpert <sup>®</sup> Dx System	Roche LightCycler	Roche LightCycler
Detection Chemistry	Paired hybridization probes using Scorpions	Paired hybridization probes using fluorescence energy transfer (FRET)	Paired hybridization probes using fluorescence energy transfer (FRET)
Fluidics/Sample Preparation	Self-contained and automated after two single-dose reagent additions.	Manual	Manual
Probes	Scorpion Probes	Hydrolysis Probe	Hydrolysis Probe
Controls	Internal Probe check control (PCC)	External positive and negative controls required per run	External positive and negative controls required per run

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

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

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

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

Guidance for the Content of Premarket Submission for Software contained in Medical Devices – Guidance for Industry and FDA Staff, May 2005

**L. Test Principle:**

The Cepheid Xpert<sup>™</sup> HemosIL<sup>®</sup> Factor II & Factor V Assay is an automated DNA test for detecting Factor II and Factor V normal and mutant alleles directly from sodium citrate or EDTA anticoagulated whole blood specimens. Blood specimens are drawn into either sodium citrate or EDTA anticoagulant tubes. Following brief mixing of the sample, the blood sample and two single-use reagents (Reagent 1 and Reagent 2) that are provided with the assay are transferred to different, uniquely-

labeled chambers of the disposable fluidic cartridge (the Xpert HemosIL Factor II & Factor V cartridge). The user initiates a test from the system user interface and places the cartridge into the GeneXpert® Dx System instrument platform, which performs hands-off real-time, multiplex polymerase chain reaction (PCR) for detection of DNA. In this platform, sample preparation, amplification, and real-time detection are all fully-automated and completely integrated.

The GeneXpert Dx System consists of a GeneXpert instrument, personal computer, a barcode scanner and the multi-chambered fluidic cartridges that are designed to complete sample preparation and real-time PCR for detection of Factor II and Factor V normal and mutant alleles in approximately 30 minutes. Each system has 1 to 16 randomly accessible modules that are each capable of performing separate sample preparation and real-time PCR tests. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing nuclei, and a proprietary I-CORE® thermocycler for performing real-time PCR and detection.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

A panel of five specimens, consisting of one of each specimen type (a wild type (NOR) sample, heterozygous Factor II/wild type Factor V, homozygous Factor II/wild type Factor V, wild type Factor II/homozygous Factor V, and a wild type Factor II/heterozygous Factor V) was tested in duplicate by two different operators on five different days at each of three sites (3 specimens x 2 times/day x 2 operators per site x 5 days x 3 sites). A different lot of Xpert HemosIL Factor II & Factor V Assay kit was used at each of the three testing sites. Xpert HemosIL Factor II & Factor V assays were performed according to the Xpert HemosIL Factor II & Factor V procedure. Results are summarized in the table below. The number of samples correctly called vs. the number of samples tested are indicated in parentheses.

**Summary of Reproducibility Results by Site – Factor II**

<b>Specimen ID</b>	<b>Site 1</b>	<b>Site 2</b>	<b>Site 3</b>	<b>% Agreement</b>
<b>NOR</b>	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
<b>Factor II HET/Factor V NOR</b>	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
<b>Factor II HOM/Factor V NOR</b>	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
<b>Factor II NOR/Factor V HOM</b>	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
<b>Factor II NOR/Factor V HET</b>	100% (20/20)	100% (20/20)	95.0% (19/20) <sup>a</sup>	98.3% (59/60) <sup>a</sup>
<b>% Agreement</b>	100% (60/60)	100% (60/60)	98.3% (59/60) <sup>a</sup>	99.7% (299/300) <sup>a</sup>

<sup>a</sup>No discordant results. One sample was indeterminate after retest.

**Summary of Reproducibility Result by Site – Factor V**

Specimen ID	Site 1	Site 2	Site 3	% Agreement
NOR	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Factor II HET/Factor V NOR	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Factor II HOM/Factor V NOR	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Factor II NOR/Factor V HOM	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Factor II NOR/Factor V HET	100% (20/20)	100% (20/20)	95.0% (19/20) <sup>a</sup>	98.3% (59/60) <sup>a</sup>
% Agreement	100% (60/60)	100% (60/60)	98.3% (59/60) <sup>a</sup>	99.7% (299/300) <sup>a</sup>

<sup>a</sup>No discordant results. One sample was indeterminate after retest.

### Summary of Reproducibility Results by Operator – Factor II

Specimen ID	Site 1		Site 2		Site 3		% Agreement
	Op 1	Op2	Op1	Op2	Op1	Op2	
NOR	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II HET/ Factor V NOR	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II HOM/ Factor V NOR	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II NOR/ Factor V HOM	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II NOR/ Factor V HET	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	90% (9/10) <sup>a</sup>	98.3% (59/60)
% Agreement	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	98% (49/50) <sup>a</sup>	99.7% (299/300) <sup>a</sup>

<sup>a</sup>No discordant results. One sample was indeterminate after retest.

### Summary of Reproducibility Result by Operator – Factor V

Specimen ID	Site 1		Site 2		Site 3		% Agreement
	Op 1	Op2	Op1	Op2	Op1	Op2	
NOR	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II HET/ Factor V NOR	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II HOM/ Factor V NOR	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II NOR/ Factor V HOM	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (60/60)
Factor II NOR/ Factor V HET	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	90% (9/10) <sup>a</sup>	98.3% (59/60)
% Agreement	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	98% (49/50) <sup>a</sup>	99.7% (299/300) <sup>a</sup>

<sup>a</sup>No discordant results. One sample was indeterminate after retest.

To assess the between lot reproducibility, the five-specimen panel described above was analyzed two times per day over five testing days using each of three assay lots at a single testing site (5 specimens x 2 runs/day x 3 lots x 5 days). A summary table of the results by lot is shown below.

**Summary of Reproducibility Results by Lot – Factor II**

<b>Specimen ID</b>	<b>Lot 1</b>	<b>Lot 2</b>	<b>Lot 3</b>	<b>% Agreement</b>
<b>NOR</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II HET/Factor V NOR</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II HOM/ Factor V NOR</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II NOR/ Factor V HOM</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II NOR/ Factor V HET</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>% Total Agreement by Lot</b>	100% (50/50)	100% (50/50)	100% (50/50)	100% (150/150)

**Summary of Reproducibility by Lot – Factor V**

<b>Specimen ID</b>	<b>Lot 1</b>	<b>Lot 2</b>	<b>Lot 3</b>	<b>% Agreement</b>
<b>NOR</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II HET/Factor V NOR</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II HOM/ Factor V NOR</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II NOR/ Factor V HOM</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>Factor II NOR/ Factor V HET</b>	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
<b>% Total Agreement by Lot</b>	100% (50/50)	100% (50/50)	100% (50/50)	100% (150/150)

b. *Linearity/assay reportable range:*

Not applicable.

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

Shelf life and open package stability testing were performed on three lots of the final product. Shelf life stability was tested using eight compound heterozygous control samples at 5±3°C; 25±3°C; 35±3°C; and 45±3°C each week for the first month, then the following two months afterwards.

Beginning with the fourth month, only the 5°C and 25°C temperatures were

tested. Shipping simulation stability studies at summer and winter temperatures were performed on one lot per package configuration. No significant difference was seen with samples tested with the assay for units stored for 3 months up to  $45\pm 3^{\circ}\text{C}$  from baseline. Real time studies beyond 25 months are ongoing, but based on 9 months of real time data and linear regression extrapolation, the recommended expiration date for the assay is 14 months when stored at  $2-28^{\circ}\text{C}$ .

d. *Detection limit:*

Analytical sensitivity - Studies were performed to determine the minimum and maximum amount of input patient specimen for both sodium citrate and EDTA anticoagulated whole blood needed to obtain a correct genotype, such that the lower bound of the 95% confidence interval for the estimated “correct call” fraction is greater than 95%. Sodium citrate and EDTA anticoagulated blood samples were tested ( $n = 20$ ) at eight volumes varying from  $5\ \mu\text{L}$  to  $250\ \mu\text{L}$ . The recommended sample volume for the Xpert HemosIL Factor II & Factor V Assay is  $50\ \mu\text{L}$ , although the assay can tolerate varying volumes from  $15\ \mu\text{L} - 100\ \mu\text{L}$ .

e. *Analytical specificity:*

To evaluate the analytical specificity of the Xpert HemosIL Factor II & Factor V Assay, normal gene sequences containing silent single nucleotide polymorphisms (SNPs) in the probe binding region as well as outside the probe binding region were synthesized. The presence of the additional SNP in the probe binding region, in most cases, resulted in an invalid result. When a valid result was obtained, it gave the correct genotype. The presence of an additional SNP outside the probe binding region resulted in the correct genotyping call.

Interfering substances – Matched EDTA and sodium citrate blood samples were tested for potential interference by the following endogenous substances: bilirubin, cholesterol, lipids and hemoglobin. The samples consisted of 11 Factor V heterozygous samples from 10 individual donors, and 11 Factor II heterozygous samples from 11 individual donors. Specimen 11 in each case was a compound heterozygote. The study compared control conditions per donor and anticoagulant (no additional interfering substance added to donor blood) to substances added at the recommended high analyte test/interference concentrations per CLSI EP7-A. The recommended interference concentrations tested were bilirubin (unconjugated) spiked at  $20\ \text{mg/dL}$ , total cholesterol spiked at  $500\ \text{mg/dL}$ , and lipids spiked at levels equivalent to or greater than  $500\ \text{mg/dL}$ . To mimic the effects of hemolysis, blood samples were stored frozen at  $-80^{\circ}\text{C}$  and then thawed before testing and comparison to the control (unfrozen/thawed) sample. The correct genotype was reported for all samples using the Xpert HemosIL FII & FV Assay. The maximum valid cycle threshold (Ct) cutoff for the Xpert HemosIL FII & FV Assay is 33.0. The analysis of interfering substances data shows that the anticoagulant has no statistical significance with p-values greater than 0.05. While some of the interfering substances show some statistical significance, the practical

significance is minimal due to distance from the Ct cutoff value. In all cases, the mean Ct remains more than 5.5 Cts below the cutoff value or more than 6.4 standard deviations below the cutoff. Studies of potentially interfering substances showed no inhibition from up to 14.3 USP units/mL heparin, 16 mg/dL bilirubin, 250 mg/dL added cholesterol, or 1932 mg/dL total triglycerides (lipids). No inhibition was observed using whole blood samples which had gone through one freeze-thaw cycle (hemolyzed blood).

f. *Assay cut-off:*  
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

Performance characteristics of the Xpert HemosIL Factor II & Factor V Assay relative to bi-directional sequencing were determined in a multi-site investigational study at seven U.S. institutions. Specimens included those whose routine care called for collection of whole blood for Factor II and Factor V testing. Samples were first tested by routine methods used in each participating laboratory and then aliquots collected for study testing by Xpert HemosIL Factor II & Factor V Assay on the GeneXpert. Excess DNA was sent to a contract laboratory for bi-directional sequencing. Performance of the Xpert HemosIL Factor II & Factor V Assay was calculated relative to bi-directional sequencing results.

A total of 1018 samples were tested for Factor II and a total of 1014 samples were tested for Factor V by both the Xpert HemosIL Factor II & Factor V Assay and by bi-directional sequencing. To supplement the homozygous sample size, six human genomic DNA samples homozygous for Factor II and five homozygous for Factor V were also tested by the Xpert HemosIL Factor II & Factor V Assay and bi-directional sequencing. The Xpert HemosIL Factor II & Factor V Assay demonstrated a 99.3% overall accuracy relative to bi-directional sequencing for both Factor II and Factor V. The results are presented in the table below.

**Xpert HemosIL Performance vs. Bi-directional Sequencing**

Genotype	Number Tested	Initial testing			Repeat testing		
		Correct calls	Invalid <sup>a</sup> (no calls)	% Agreement	Correct calls	Invalid <sup>a</sup> (no calls)	% Agreement
<b>Factor II G20210A</b>							
WT	968	927	41	95.80%	963	5	99.50%
HET	50	48	2	96.00%	48	2	96.00%
HOM	7	7	0	100.00%	7	0	100%
Overall	1025 <sup>b</sup>	982	43	95.80%	1018	7	99.30%
<b>Factor V G1691A</b>							
WT	895	860	35	96.10%	889	6	99.30%
HET	114	108	6	94.70%	113	1	99.10%
HOM	12	11	1	91.70%	12	0	100.00%
Overall	1021 <sup>c</sup>	979	42	95.90%	1014	7	99.30%

- <sup>a</sup>No discordant results. Invalid results refer to “indeterminate” results.
- <sup>b</sup>Bi-directional sequencing results for Factor II were not available for 4 specimens.
- <sup>c</sup>Bi-directional sequencing results for Factor V were not available for 8 specimens.

- b. Matrix comparison:*  
Matched sodium citrate or EDTA samples were compared and no statistical difference was observed.
- 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:  
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.