Cepheid®

Instructions for Use

Xpert® Factor II & Factor V

For use with GeneXpert® System with Touchscreen

Catalog Numbers

REF GXFIIFV-10

302-9618 | Rev. C | 2024-05

℞only IVD *In Vitro* Diagnostic Medical Device

Trademarks, Patents, and Copyright Statements

See Revision History for a description of changes.

Table of Contents

Getting Started 5
Product Information 5
Proprietary Name 5
Common or Usual Name 5
Intended Use, Summary, and Principle of Procedure 5
Intended Use 5
Summary and Explanation 5
Principle of the Procedure 6

Reagents, Instruments, and Materials 7
Reagents 7
Materials Provided 7
Materials Required but Not Provided 7
Warnings and Precautions 8
Chemical Hazards, Storage and Handling 9
Chemical Hazards 9
Storage and Handling 9

Specimen Collection, Testing, and Results 11
Specimen Collection 11
Specimen Collection, Transport, and Storage 11
Procedure 11
Preparing the Cartridge 11
Starting the Test: GeneXpert System with Touchscreen 12
Viewing Results: GeneXpert System with Touchscreen 13
Quality Control 13
Results 14
Reasons to Repeat the Test 14
Limitations 14
Limitations of the Procedure 15
Expected Values 15

Specific Performance Characteristics 16
Clinical Performance 16
Xpert Factor II & Factor V Test 16
Analytical Performance 17
Analytical Sensitivity 17
Analytical Specificity 17
Interfering Substances 17
Reproducibility 17

Appendix 21
Bibliography 21
Cepheid Headquarters Locations 21
Technical Assistance 22
Table of Symbols 22
Revision History 23

Getting Started

Product Information

Proprietary Name

Xpert® FII & FV

Common or Usual Name

Xpert Factor II & Factor V

Intended Use, Summary, and Principle of Procedure

Intended Use

The Xpert FII & FV test 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 test is performed on the Cepheid GeneXpert® Instrument Systems. 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.

Summary and Explanation

The association of Factor II (G20210A) and Factor V Leiden (G1691A) mutations with an increased risk for venous thrombosis has been well documented.[1,2,3,4] Factor II c.*97G>A was previously designated as G20210A or 20210G>A4 and is commonly referred to as prothrombin or, as in the Xpert Factor II & Factor V test, as Factor II (G20210A). The Factor II (G20210A) mutation refers to the G to A transition at nucleotide 20210 in the 3' untranslated region of the gene and is associated with increased plasma levels of prothrombin.

Factor V c.1601G>A (p.Arg534Gln) was previously designated as G1691A or Arg506Gln and is commonly referred to as Factor V Leiden or FVL[5], or as in the Xpert Factor II & Factor V test, as Factor V (G1691A). Factor V Leiden (G1691A) refers to the G to A transition at nucleotide position 1691 of the Factor V gene, resulting in the substitution of the amino acid arginine by glutamine in the Factor V protein, causing resistance to cleavage by Activated Protein C (APC).

Factor II (G20210A) and Factor V Leiden (G1691A) mutations are present in 2% and 5% of the general population, respectively.[6]

Principle of the Procedure

The GeneXpert Instrument System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in whole blood using real-time Polymerase Chain Reaction (PCR) tests. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests and viewing the 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. For a full description of the system, see the relevant system operator manual.

The Xpert Factor II & Factor V test includes reagents for the detection of Factor II and Factor V normal and mutant alleles from sodium citrate or EDTA anticoagulated whole blood. Each test cartridge also contains a Probe Check Control (PCC) that verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers and probes in the Xpert Factor II & Factor V test determine the genotype of the Factor II gene (at position 20210) and/or the Factor V gene (at position 1691).

Reagents, Instruments, and Materials

Reagents

Materials Provided

The Xpert Factor II & Factor V test kit contains sufficient reagents to process 10 specimens or quality control samples.

The kit contains the following:

Xpert Factor II & Factor V test Cartridges with integrated reaction tubes	**10**
Bead 1 and Bead 2 (freeze-dried)	**1 of each per cartridge**
Reagent 1	**3.0 mL per cartridge**
Reagent 2 (Guanidinium Hydrochloride)	**3.0 mL per cartridge**
CD	**1 per kit**

- Assay Definition Files (ADF)
- Instructions to import ADF into GeneXpert software
- Instructions for Use (Package Insert)

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

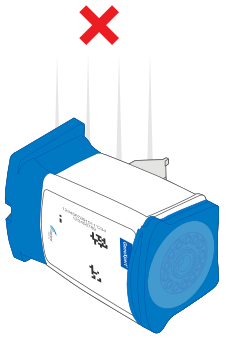
Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

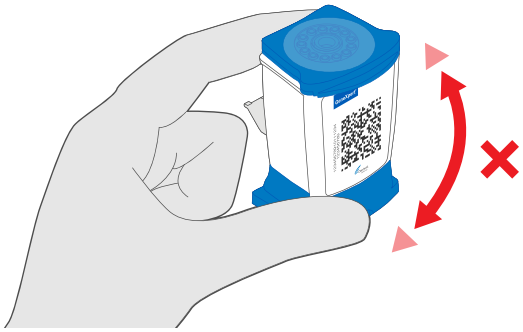
Materials Required but Not Provided

- GeneXpert system with touchscreen: GeneXpert instrument, touchscreen unit with built-in scanner, Cepheid OS software version 2.0 or higher, and operator manual.
- Pipette to dispense 50 µL sodium citrate or EDTA anticoagulated blood with aerosol-resistant filter tips.
- HemosIL FII & FV DNA Control, P/N 0020003500.

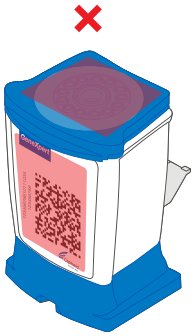
Warnings and Precautions

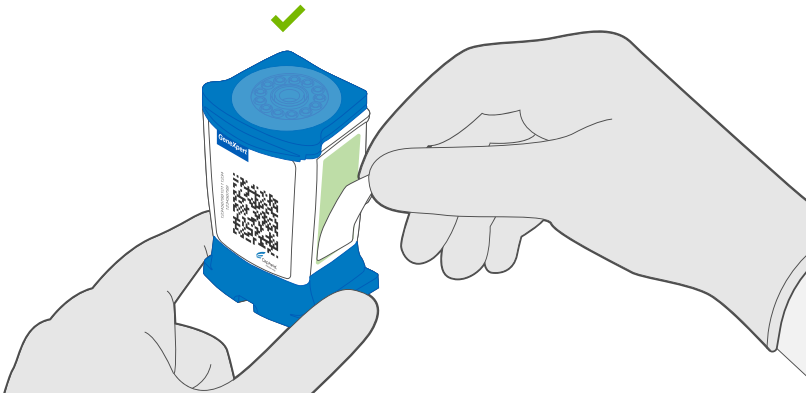
- For *in vitro* Diagnostic Use. Rx only
- Do not use a cartridge that has been dropped after removing from the kit or that has been shaken after the cartridge lid has been opened. Shaking or dropping the cartridge after opening the lid may yield false or non-determinate results.



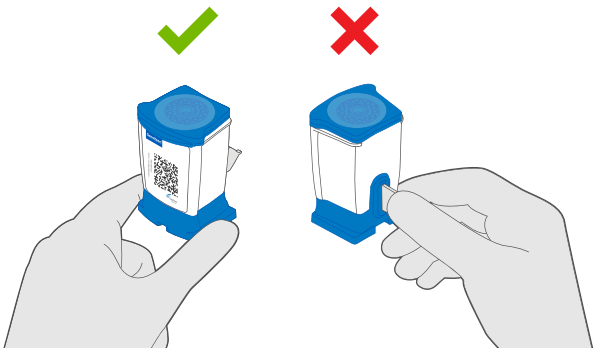


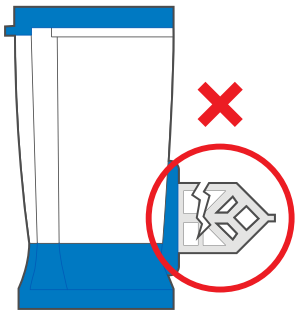
- Do not place the sample ID label on the cartridge lid or on the barcode label.





- Hold the cartridge by the base. Do not touch the reaction tube at the rear of the cartridge as this could cause damage that would interfere with light passing through it during the test. Do not use a cartridge with a damaged reaction tube.





- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[7] and the Clinical and Laboratory Standards Institute[8] .
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Use the cartridges before the expiration date indicated on the kit.

- Do not open the Xpert Factor II & Factor V test cartridge lid except when adding sample.
- Each single-use Xpert Factor II & Factor V test cartridge is used to process one test. Do not reuse spent cartridges.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.
- Store the Xpert Factor II & Factor V test kit at 2-28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- In the event the internal pressure rises above the pre-set manufacturer limit, the run will automatically abort and an **ERROR** result will be reported.

Chemical Hazards, Storage and Handling

Chemical Hazards[9,10]

- UN GHS Hazard Pictogram: 
- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - May be harmful if swallowed
  - Causes skin irritation
  - Causes serious eye irritation
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash thoroughly after handling.
    - Wear protective gloves/protective clothing/eye protection/face protection
  - **Response**
    - IF ON SKIN: Wash with plenty of soap and water.
    - Specific treatment, see supplemental first aid information.
    - If skin irritation occurs: Get medical advice/attention
    - Take off contaminated clothing and wash before reuse.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention
    - Call a POISON CENTER or doctor/physician if you feel unwell.

Storage and Handling

- Store the Xpert Factor II & Factor V test cartridges at 2 – 28 °C.
- Do not use cartridges that have passed the expiration date.
- Do not open a cartridge until you are ready to perform testing.

- Use the cartridge and reagents within 30 minutes after opening the cartridge lid.

Specimen Collection, Testing, and Results

Specimen Collection

Specimen Collection, Transport, and Storage

To obtain adequate specimen, follow the instructions in this section closely.

- Only trained, licensed professionals should draw blood in EDTA or sodium citrate anticoagulant tubes.
- Do not centrifuge or concentrate the blood sample by plasma removal.
- Blood should be processed within 24 hours when stored at room temperature (22-28 °C). Samples should be stored at 2-8 °C if stored longer than 24 hours. Blood is stable up to 15 days when stored at 2-8 °C. The blood samples may also be stored at -20 °C or -80 °C for up to 3 months. Use of a freezer-compatible storage vial is recommended.

Note Allow frozen blood to thaw completely at room temperature. It is not recommended to freeze/thaw blood more than one time.

- Mix sample by inverting 5 times prior to dispensing into the cartridge

Procedure

Preparing the Cartridge

Important Start the test within 15 minutes of adding the sample to the cartridge.

To add the sample into the cartridge:

1. Remove the cartridge from the kit. It is not necessary to bring the cartridge to room temperature before use.
2. Mix sample by inverting the tube at least 5 times, until homogeneous.
3. Open the cartridge lid. Using a pipette with an aerosol resistant tip, transfer 50 µL of sodium citrate or EDTA anticoagulated blood to the bottom wall of the Sample opening of the Xpert Factor II & Factor V test cartridge. See Figure 1.
4. Close the cartridge lid.

Figure 1 Xpert Factor II & Factor V Cartridge

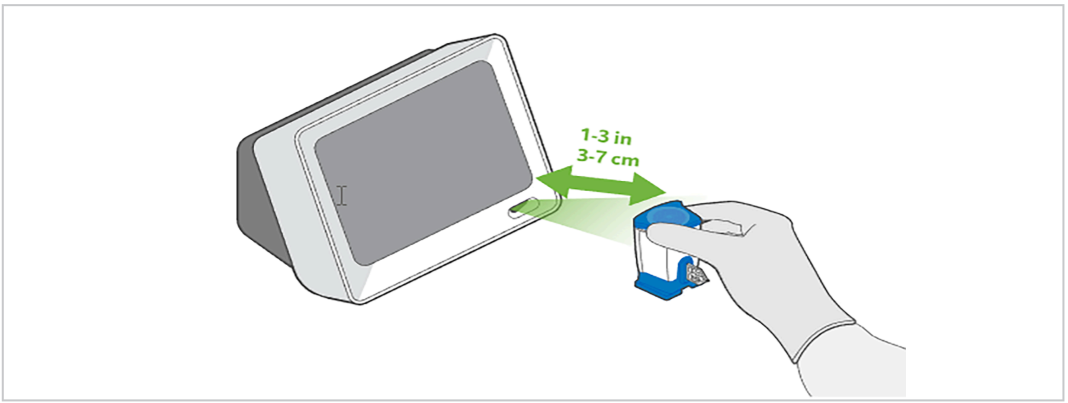
Starting the Test: GeneXpert System with Touchscreen

Important **Before you start the test, make sure that:**

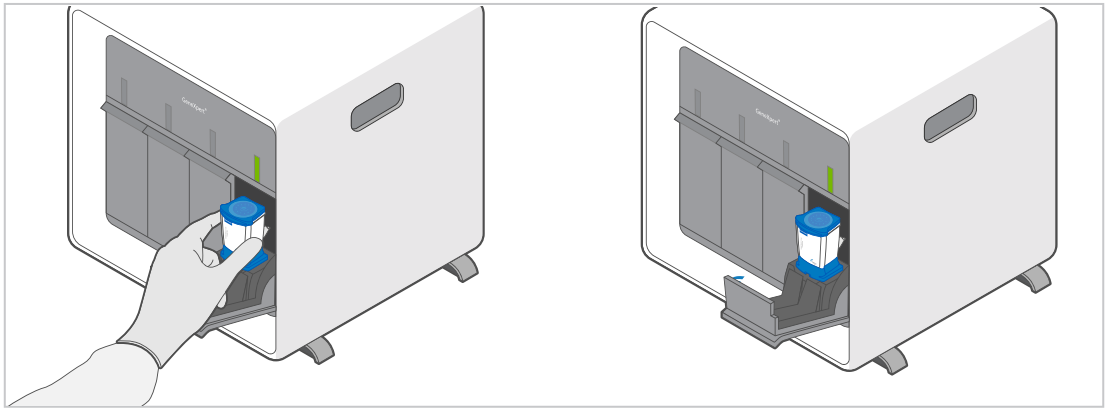
- **The system is running the correct Cepheid OS software version shown in section - Materials Required but Not Provided.**
- **The correct assay definition file is imported into the software.**

Note The default workflow is shown. Your system administrator may alter the workflow.

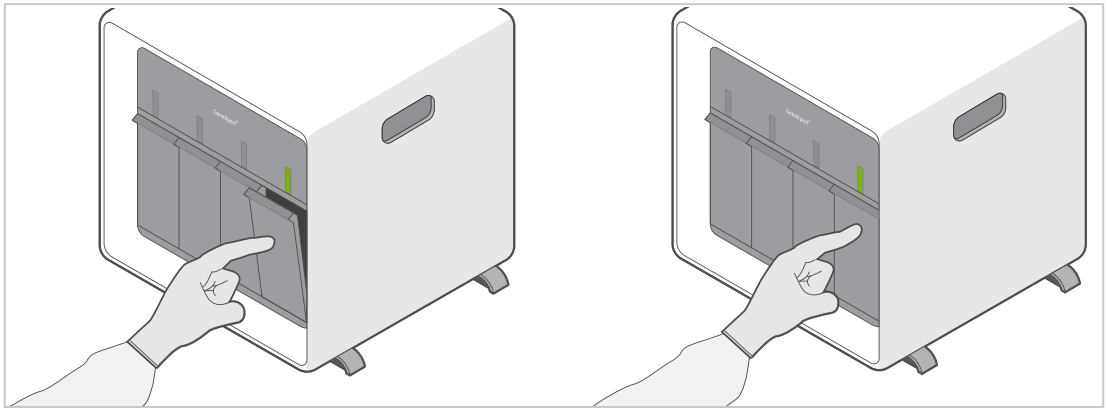
1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test**.
4. Follow onscreen prompts to create new test and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If scanning, hold the cartridge about 1-3 inches (3-7 cm) away from the scanner. The scanner projects a green crosshair, which you center on the barcode. Scanning is complete when you hear an audible beep. Touch **Continue**.



6. Select the desired test and touch **Continue**.
7. Watch the cartridge preparation video, if needed.
8. On the Confirm screen, review all data and touch **Confirm**.
9. Open the module door under flashing green light and insert the cartridge.



10. Close cartridge module door completely by pressing until it latches. The test starts.



11. When the test completes, the **Results Summary** screen appears. Open the module door and remove cartridge.
12. Dispose of used cartridge in appropriate waste container according to your institution's standard practices.

Viewing Results: GeneXpert System with Touchscreen

The GeneXpert system with touchscreen results screen will automatically interpret test results for you and clearly show them in the **View Results** window.

1. Tap **Results**.
2. Tap the test to be viewed in the Results screen.
3. Click **OK**.
4. To generate a PDF report file, touch **View Report**. More detailed instructions for viewing and uploading results are available in your system operator manual.

Quality Control

Each test includes a probe check (PCC).

Probe check control (PCC) - Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

External Controls - External quality controls are not required but may be used in accordance with local, state,

and federal accrediting organizations, as applicable. For example, Normal, heterozygous, or homozygous Factor II/Factor V whole blood samples (sodium citrate or EDTA anticoagulant) may be used as external controls in the Xpert Factor II & Factor V test. Cell-based material is recommended, extracted DNA is not suitable control material. Verification of the selected external controls is recommended prior to use.

Results

The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded algorithms to identify genotypes, and are shown in the following View Results windows:

Table 1. Possible Results

Result	Interpretation
NORMAL	The result 'NORMAL' refers to wildtype (no mutation detected).
HOMOZYGOUS	The result 'HOMOZYGOUS' refers to 'homozygous mutant' (mutation detected in both alleles).
HETEROZYGOUS	The result "HETEROZYGOUS' refers to 'heterozygous mutant' (mutation detected in one allele).
INVALID	Presence or absence of Factor II/Factor V normal and mutant alleles cannot be determined; repeat test according to instructions below. The sample was not properly processed or PCR was inhibited. • **INVALID**—Presence or absence of Factor II/Factor V normal and mutant alleles cannot be determined. • Probe Check—PASS; all probe check results pass.
ERROR	Presence or absence of Factor II/Factor V normal and mutant alleles cannot be determined; repeat test according to instructions below. The Probe Check control failed and the test aborted possibly due to an improperly filled reaction tube, or a probe integrity problem was detected. Errors may also be caused by exceeding the maximum pressure limits or a system component failure. • **ERROR** • Probe Check—FAIL*; one or more of the probe check results fail. *If the probe check passed, the error is caused by a system component failure.
NO RESULT	Presence or absence of Factor II/Factor V normal and mutant alleles cannot be determined; repeat test according to instructions below. Insufficient data were collected to produce a test result (for example, this can occur if the operator stopped a test that was in progress). • **NO RESULT** • Probe Check—NA (not applicable)

Reasons to Repeat the Test

Repeat the test using a new cartridge (do not re-use the cartridge) and a new aliquot of sodium citrate or EDTA anticoagulated whole blood:

- An **INVALID** result indicates that the sample was not properly processed or PCR was inhibited.
- An **ERROR** result indicates that the Probe Check control failed and the test was aborted possibly due to an improperly filled reaction tube, or a reagent probe integrity problem was detected. Errors may also be caused by exceeding the maximum pressure limits or a system component failure.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Limitations

Limitations of the Procedure

- The performance of the Xpert Factor II & Factor V test was validated using the procedures provided in this instructions for use only. Modifications to these procedures may alter the performance of the test. Results from the Xpert Factor II & Factor V test should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Rare Factor V mutations (A1696G, G1689A, and A1692C) and any additional SNPs in the probe binding region may interfere with the target detection and yield an INVALID result.
- Other rare Factor II mutations in the probe binding region may interfere with the target detection and could yield an INVALID result, or a false HOMOZYGOUS mutant result when occurring concordantly with the Factor II c.*97G>A (G20210A) mutation.
- The performance of the Xpert Factor II & Factor V test has not been evaluated with samples from pediatric patients.
- Erroneous test results might occur from improper specimen collection, handling, or storage or sample mix-up. Careful compliance to the instructions in this package is necessary to avoid erroneous results.

Expected Values

Factor II (G20210A) and Factor V Leiden (G1691A) mutations are present in 2% and 5% of the general population, respectively[6].

Specific Performance Characteristics

Clinical Performance

Performance characteristics of the Xpert Factor II & Factor V test were determined in a multi-site investigational study at seven institutions by comparing the Xpert Factor II & Factor V test with bi-directional sequencing.

Specimens included those whose routine care called for collection of whole blood for Factor II and/or Factor V testing. Samples were first tested by routine methods used in each participating laboratory and then aliquots collected for study testing by the Xpert Factor II & Factor V test on the GeneXpert. Excess DNA was sent to a contract laboratory for bi-directional sequencing.

Performance of the Xpert Factor II & Factor V test was calculated relative to bi-directional sequencing results.

Xpert Factor II & Factor V Test

A total of 1018 samples were tested for Factor II by both the Xpert Factor II & Factor V test and bi-directional sequencing. A total of 1014 samples were tested for Factor V by both the Xpert Factor II & Factor V test and 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 Factor II & Factor V test and bi-directional sequencing. The results are presented in Table 2.

The Xpert Factor II & Factor V test demonstrated a 99.3% overall accuracy relative to bi-directional sequencing for both Factor II and Factor V.

Table 2. Xpert Factor II & Factor V Test Performance vs. Bi-directional Sequencing

Genotype	Number Tested	Number of Correct Calls on First Run	Number of Invalid[a] Calls on First Run	Agreement on First Run	Number of Correct Calls Including Repeat Run	Number of Invalid[a] Calls on Repeat Run	Agreement After Repeat Run
Factor II G20210A							
WT[b]	968	927	41	95.8%	963	5	99.5%
HET	50	48	2	96.0%	48	2	96.0%
HOM	7	7	0	100.0%	7	0	100%
Overall	1025[c]	982	43	95.8%	1018	7	99.3%
Factor V G1691A							

Genotype	Number Tested	Number of Correct Calls on First Run	Number of Invalid[a] Calls on First Run	Agreement on First Run	Number of Correct Calls Including Repeat Run	Number of Invalid[a] Calls on Repeat Run	Agreement After Repeat Run
WT	895	860	35	96.1%	889	6	99.3%
HET	114	108	6	94.7%	113	1	99.1%
HOM	12	11	1	91.7%	12	0	100.0%
Overall	1021[d]	979	42	95.9%	1014	7	99.3%

a. No discordant results. Invalid results refer to "indeterminate" results

b. WT (wildtype) is normal

c. Bi-directional sequencing results for Factor II were not available for 4 specimens

d. Bi-directional sequencing results for Factor V were not available for 8 specimens

Analytical Performance

Analytical Sensitivity

Studies were performed to determine the minimum and maximum amount of input patient specimen for both EDTA and sodium citrate 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%.

EDTA and sodium citrate anticoagulated blood samples were tested (n=20) at 8 volumes varying from 5 µL to 250 µL.

Although the test can tolerate varying volumes from 15 µL - 100 µL, 50 µL is the recommended sample volume to minimize the risk of errors associated with limited and excess sample.

Analytical Specificity

To evaluate the analytical specificity of the Xpert Factor II & Factor V test, 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

Patients on heparin therapy and blood transfusion patients may have blood specimens that potentially interfere with the PCR results and lead to invalid or erroneous results.

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). No statistical significance was observed between matched specimens drawn into EDTA or sodium citrate.

Reproducibility

A panel of 5 specimens, consisting of one of each specimen type listed below, was tested in duplicate by two

different operators on 5 different days at each of three sites (3 specimens x 2 times/day x 2 operators per site x 5 days x 3 sites). One lot of XpertFactor II & Factor V test kit was used at each of the 3 testing sites. Xpert Factor II & Factor V tests were performed according to the Xpert Factor II & Factor V procedure. Results are summarized in Table 3 through Table 6.

Study panel:

1. a sample with normal (wildtype) alleles for both Factor II & Factor V;
2. a sample heterozygous for Factor II mutation (i.e., one mutant and one wildtype allele for Factor II gene) and with normal (wildtype) alleles for Factor V;
3. a sample homozygous for Factor II mutation (i.e., two mutant alleles for Factor II gene) and with normal (wildtype) alleles for Factor V;
4. a sample with normal (wildtype) alleles for Factor II and homozygous for Factor V mutation (i.e., two mutant alleles for Factor V gene);
5. a sample with normal (wildtype) alleles for Factor II and heterozygous for Factor V mutation (i.e., one mutant and one wildtype allele for Factor V gene).

A summary of the results by site is shown in Table 3 and Table 4. There was no statistically significant difference in results among sites for either Factor II (p=1.000) or Factor V (p=1.000).

Table 3. Summary of Reproducibility Results by Site - Factor II

Specimen ID	Site 1	Site 2	Site 3	% Total Agreement by Sample
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)[a]	98.3% (59/60)[a]
% Total Agreement by Site	100% (60/60)	100% (60/60)	98.3% (59/60)[a]	99.7% (299/300)[a]

a. No discordant results. One sample was indeterminate after retest.

Table 4. Summary of Reproducibility Results by Site - Factor V

Specimen ID	Site 1	Site 2	Site 3	% Total Agreement by Sample
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)[a]	98.3% (59/60)[a]
% Total Agreement by Site	100% (60/60)	100% (60/60)	98.3% (59/60)[a]	99.7% (299/300)[a]

a. No discordant results. One sample was indeterminate after retest.

A summary of the results by operator is shown in Table 5 and Table 6. There was no statistically significant difference in results among sites for either Factor II (p=1.000) or Factor V (p=1.000).

Table 5. Summary of Reproducibility Results by Operator - Factor II

Specimen ID	Site 1		Site 2		Site 3		% Total Agreement by Sample
	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2	
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)	90.0% (9/10)[a]	98.3% (59/60)[a]
% Total Agreement by Operator	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	98.0% (49/50)[a]	99.7% (299/300)[a]

a. No discordant results. One sample was indeterminate after retest.

Table 6. Summary of Reproducibility Results by Operator - Factor V

Specimen ID	Site 1		Site 2		Site 3		% Total Agreement by Sample
	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2	
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)	90.0% (9/10)[a]	98.3% (59/60)[a]
% Total Agreement by Operator	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)	98.0% (49/50)[a]	99.7% (299/300)[a]

a. No discordant results. One sample was indeterminate after retest.

To assess the between lot reproducibility, the 5-specimen panel described above was analyzed two times per day over 5 testing days using each of three test lots at a single testing site (5 specimens x 2 runs per day x 3 lots x 5 days). A summary of the results by lot is shown in Table 7 and Table 8. There was no statistically significant difference in results between lots for either Factor II (p=1.000) or Factor V (p=1.000).

Table 7. Summary of Reproducibility Results by Lot - Factor II

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

Table 8. Summary of Reproducibility Results by Lot - Factor V

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

Appendix

Bibliography

1. Thrombophilia as a multigenic disease. B. Zoeller, P.G. de Frutos, A. Hillarp, B. Dahlback. Haematologica 1999; 84:59–70.
2. Screening for inherited thrombophilia: indications and therapeutic implications. V. De Stefano, E. Rossi, K. Paciaroni, G. Leone. Haematologica 2002; 87:1095 – 1108.
3. Laboratory investigation of thrombophilia. A Tripodi and P.M. Mannucci. Clinical Chemistry 2001; 47:1597–1606.
4. Zhang et al. Venous thromboembolism laboratory testing (factor V Leiden and factor II c.*97G>A), 2018 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine (2018) 20:1489–1498
5. Montagnana M, Lippi G, Danese E.An Overview of Thrombophilia and Associated Laboratory Testing. Methods Mol Biol. 2017;1646:113-135
6. Grody WW, Griffin JH, Taylor AK, *et al.* American college of medical genetic consensus statement on factor V leiden mutation testing. Genetics in Medicine. 2001; 3(2):139–148.
7. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. 5th Edition HHS Publication No. (CDC) 21-1112 Revised December 2009 https://www.cdc.gov/labs/BMBL.html.
8. Clinical and Laboratory Standards Institute document M29-A4—Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline 4th Edition. 2014
9. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
10. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive

Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

United States Technical Support

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France Technical Support

Telephone: + 33 563 825 319
Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse

Symbol	Meaning
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
	For prescription use only

Manufacturer Information

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

IVD

Revision History

Description of Changes: 302-9618 Rev. B to C

Purpose: Update of Intended Use statement

Section	Description of Change
Intended Use	Revised Intended Use statement