Assay Training:
Xpert® FII & FV Training

Technical Training for US-IVD and CE-IVD product only

301-2118 Rev. C October 2018

CE-IVD and US-IVD. For *in vitro* diagnostic use.

Training Agenda

- Xpert FII & FV Training
  - Clinical utility
  - Kit storage and handling
  - Specimen collection, transport, and storage
  - Preparing cartridge
  - Assay targets
  - Result analysis
  - Quality Control
  - Discussion and Q&A

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Training Objectives

At the end of the training, users will be able to:

- Store and handle the Xpert FII & FV materials.
- Follow proper laboratory safety precautions.
- Collect appropriate specimen types and transport specimen.
- Perform the cartridge set up and run the assay.
- Report the various software generated results.
- Discard the used materials.

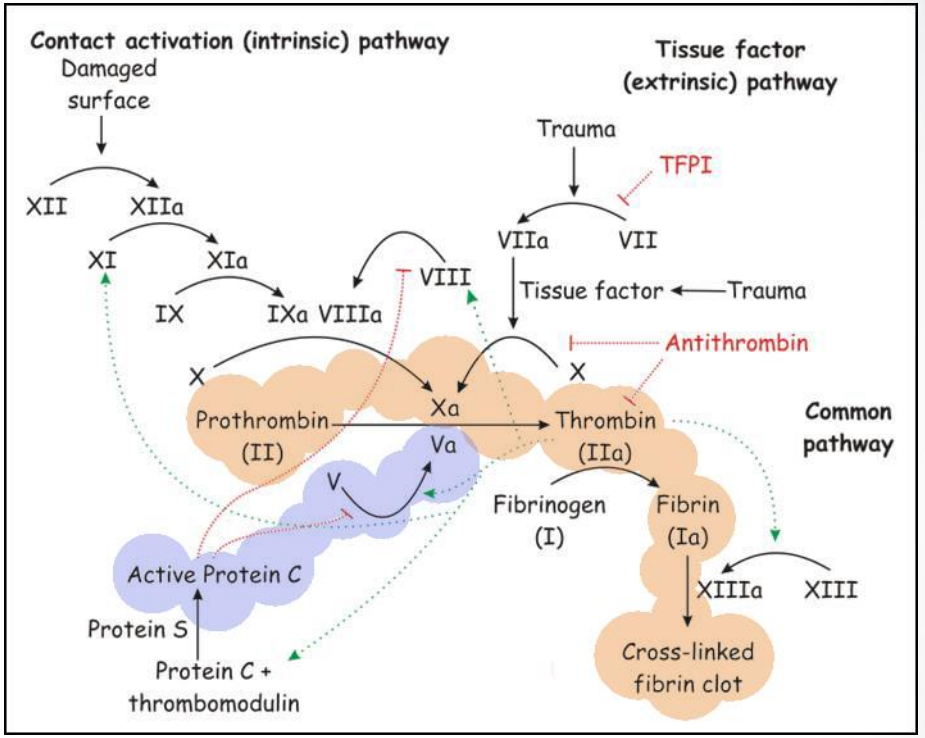
FII & FV Leiden

What are FII & FV Leiden mutations?

- Factor II (G20210A) and Factor V Leiden (G1691A) mutations are associated with an increased risk for venous thrombosis.
- These mutations are respectively present in 2% and 5% of the general population.

- Factor II or Prothrombin (G20210A) mutation
  - G to A transition at nucleotide 20210 in the 3' untranslated region of the gene
  - associated with increased plasma levels of prothrombin

- Factor V Leiden (G1691A) mutation
  - 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).



Genotypes

	Normal	Heterozygous	Homozygous (mutant)
Factor II	GG	GA	AA
Factor V	GG	GA	AA

The Cepheid Solution

- Simultaneous detection
  - Factor II and Factor V normal and mutant alleles
- One internal control for each individual sample
  - Probe Check Control (PCC)
- Results in approximately 30 minutes
- Simple and easy to use
  - Closed cartridge system
- On-demand results 24/7
- Random access

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Intended Use

The Xpert® 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 test is performed on the Cepheid GeneXpert® Dx System software version 4.0 or higher.

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.

System and Reagent Requirements

GeneXpert Systems

- 6 color modules
- GXDX or Xpertise Software v4.0 or higher

Test Kits

- GXFIIFV-10

Sample Collection

- Whole blood in EDTA or Sodium Citrate collection tube

Materials Required but not Provided

- Pipette to dispense 50 µL sodium citrate or EDTA anticoagulated blood with aerosol-resistant filter tips.
- Bleach
- 70% ethanol or denatured ethanol

Xpert FII & FV Kit Components

Tests per kit	10
Kit CD	Assay Definition Files (ADF)
	Assay Import Instructions
	Package insert
Storage	2-28˚C

Lysis Reagent contains guanidinium thiocyanate (H302, H316, H320, H402, EUH031), which is harmful if swallowed, caused mild skin irritation, causes eye irritation, is harmful to aquatic life, and contact with acid liberates toxic gas.

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Xpert FII & FV Specimen Transport and Storage

Specimen	Transport and Storage Temperature (˚C)	Storage Time
Whole Blood in EDTA  or Whole Blood in sodium citrate anticoagulant tubes 	2-8 ˚C	15 days
	22-28 ˚C (Room Temperature)	24 hours
	-20 ˚C or -80 ˚C	3 months

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

Good Laboratory Practice

PCR laboratory setup	• Cartridge/reagent preparation → Sample addition → Detection
Specimen and reagent storage	• Store specimens separately from reagents to prevent reagent contamination.
Equipment	• Use filtered pipette tips, when needed. • Follow the manufacturer's recommendation for calibration and maintenance of the lab equipment. • Perform regular maintenance on the GeneXpert Instrument.

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Good Laboratory Practice, continued

Housekeeping	• Clean work surfaces with a 1:10 dilution of household bleach* in water and then a 70% ethanol solution. Wipe work surfaces dry.
Personnel	• Wear clean lab coats and gloves. • Change gloves between processing samples.
Lab bench area	• Clean the lab bench area routinely. • Keep the back of the instrument dust free.

** Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration in your country*

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Xpert FII & FV Kit Storage and Handling

- Store test kits at 2-28ºC. Do not use expired cartridges.
- Each single-use cartridge is used to process one test. Do not reuse processed cartridges.
- Do not open a cartridge until ready to use.
  - Start the test within 15 minutes of adding the sample to the cartridge.
- Avoid cross contamination during sample handling steps.
  - Change gloves if they come in contact with specimen or appear to be wet.
  - Change gloves before leaving work area and upon entry into work area.
- Do not use a cartridge that has been dropped or shaken after the sample has been transferred to the cartridge. Shaking or dropping the cartridge after opening the lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use a cartridge that has leaked.

Xpert FII & FV Cartridge Preparation

Xpert® Factor II & Factor V Cartridge Preparation

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

Cepheid Technical Support

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+33 563 82 53 19
support@cepheideurope.com

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A better way.

1 Obtain the EDTA or sodium citrate sample collection tube.

2 Obtain one Xpert Factor II & Factor V cartridge.

3 Gently mix sample by inverting the sample tube 5 times, until homogeneous.

4 Open the Xpert cartridge lid.

5 Using a volumetric pipette with an aerosol resistant tip, aspirate 50ul. of the sample.

6 Transfer the sample to the bottom wall of the "S" chamber of the cartridge.

7 Close the Xpert cartridge lid.

8 Load the cartridge and start the assay.

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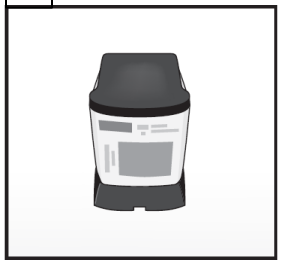
FII & FV Cartridge Preparation

1

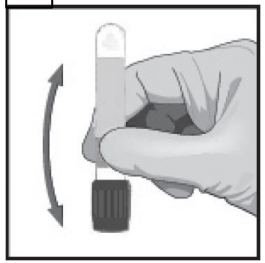
Obtain the EDTA or sodium citrate sample collection tube.

2



Obtain one Xpert Factor II & Factor V cartridge.

3

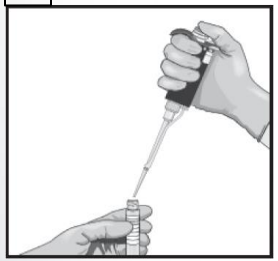


Gently mix sample by inverting the sample tube 5 times, until homogenous.

4

Open the Xpert cartridge lid.

5



Using a volumetric pipette with an aerosol resistant tip, aspirate 50µL of the sample.

6

Transfer the sample to the bottom wall of the "S" chamber of the cartridge.

7

Close the Xpert cartridge lid.

8

Start the test within the timeframe specified in the package insert.

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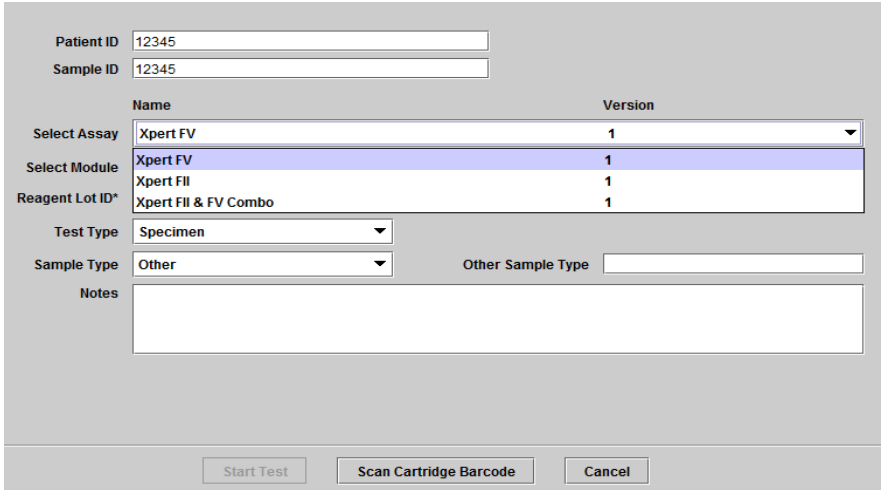
Combinatorial reporting: Xpert FII & FV ADF

- The combinatorial function in the GX v4.0 software or higher allows the operator to select one of three assay reporting options:
  - "Xpert FII", "Xpert FV", or "Xpert FII & FV Combo"



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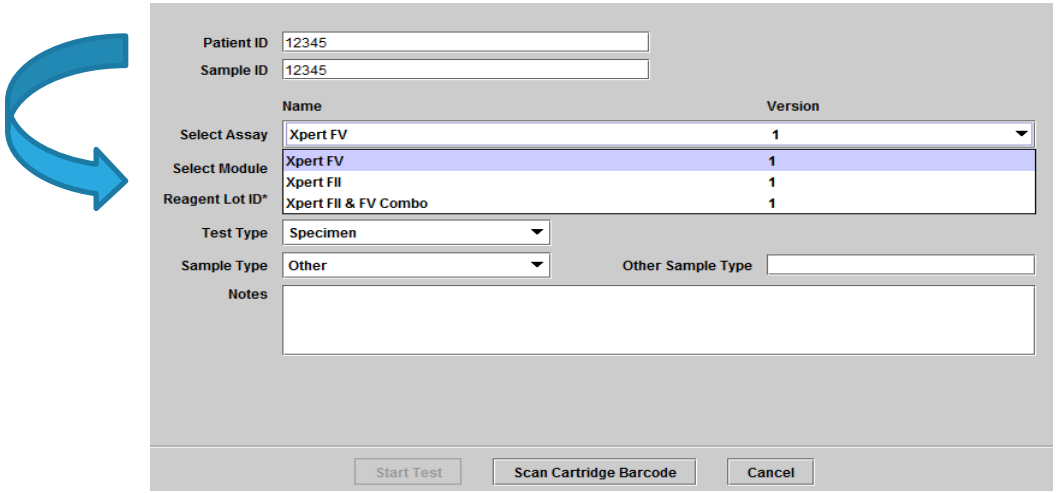
Combinatorial reporting: Xpert FII & FV ADF

- Choose the desired test from the “Select Assay” drop-down menu



- Only the test result for the assay selected at this step will be collected once the test is started.
  - Example: If the operator selects the “Xpert FII”, once the assay starts, the option cannot be changed to collect FII and FV data

Automated Xpert FII & FV Test Steps

1. Add the sample to the cartridge
2. Place the cartridge into the instrument.
3. Nucleic acids are purified.
4. Purified nucleic acids mix with PCR reagents.
5. Simultaneous amplification and detection occurs.
6. Results are ready to view.

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Quality Control

Refer to the Package Insert for complete details

Cepheid Assay Control Strategy

- Each Xpert cartridge is a self-contained test device.
  - Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge.
    - Instrument system control: Check status
    - Endogenous control
    - Reagent control: Probe Check

Instrument System Control – Check Status

- System control checks the optics, temperature of the module, and mechanical integrity of each cartridge.
  - If the system controls fail, an ERROR test result will be reported.

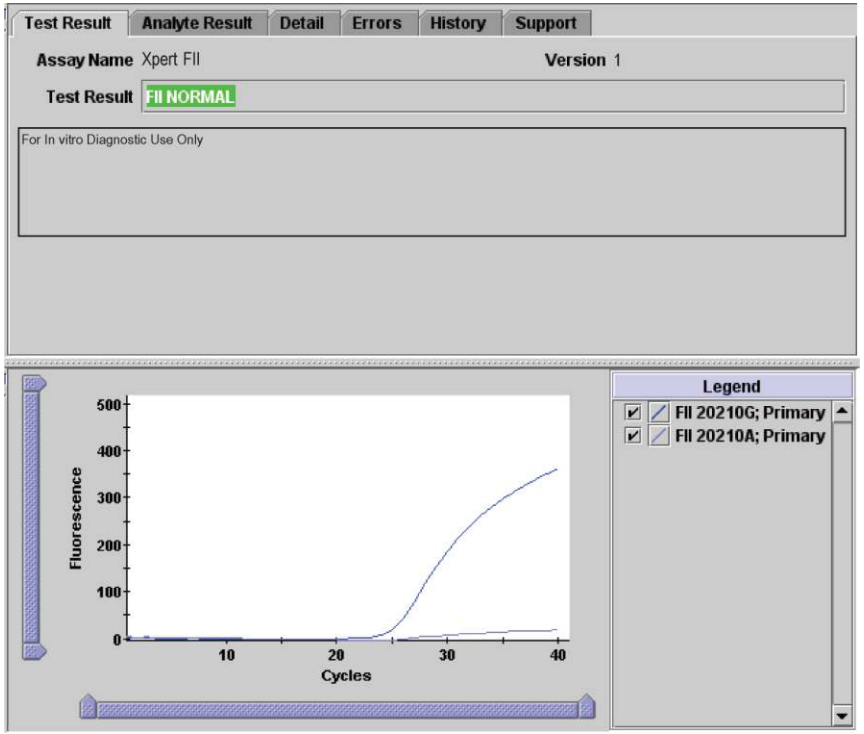
Probe Check Control - PCC

- Before the start of the PCR reaction, the fluorescence signal is measured from the probe to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability.
- The readings are compared to default settings established by Cepheid.
- The Probe Check controls for:
  - Missing Target Specific Reagent (TSR) and/or Enzyme Reagent beads, which contain all primers, probes, and internal control template
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Probe degradation
- The PCC passes if it meets the assigned acceptance criteria.
- If the Probe Check fails, an ERROR test result will be reported.

Results Analysis

Refer to the Package Insert for complete details

Xpert FII Normal Result



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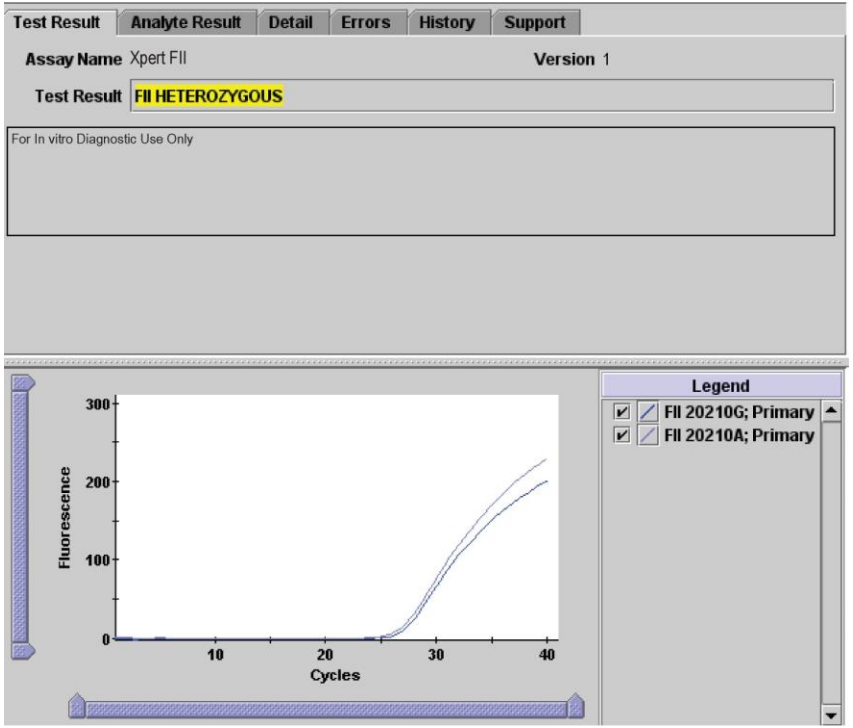
Xpert FII Heterozygous Result



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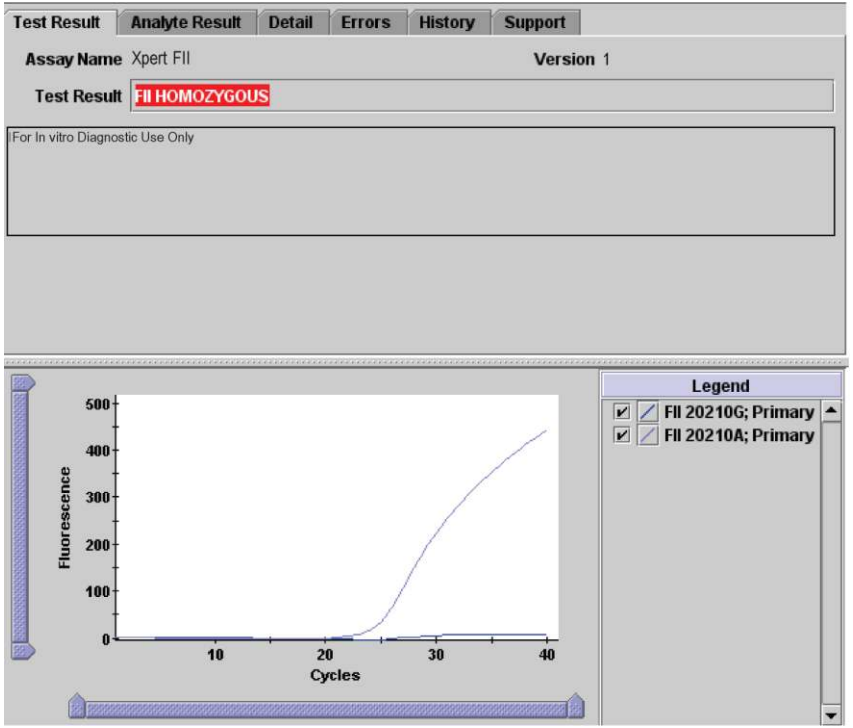
Xpert FII Homozygous Result



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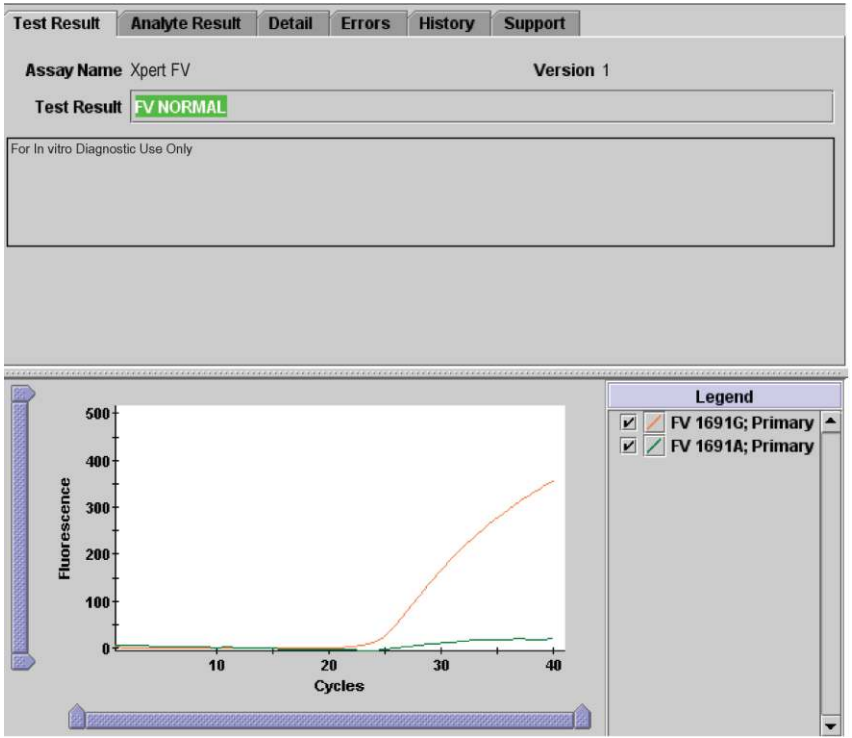
Xpert FV Normal Result



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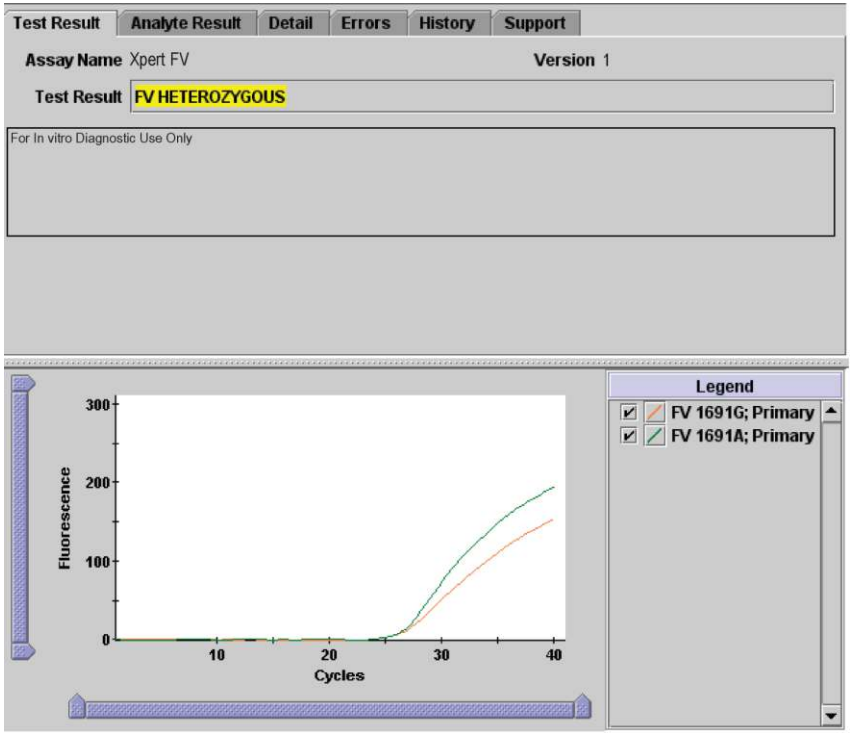
Xpert FV Heterzygous Result



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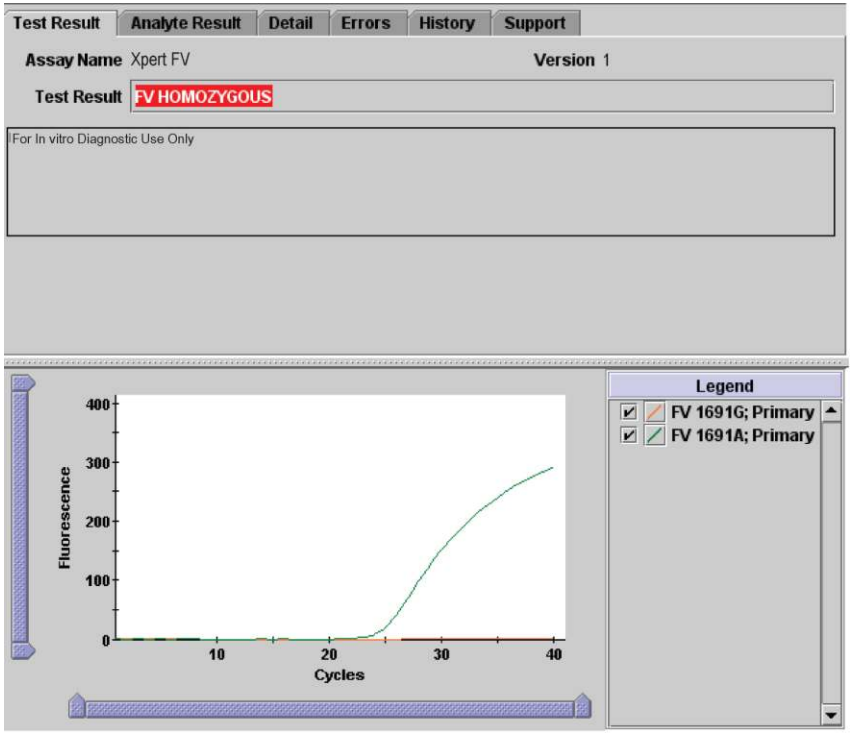
Xpert FV Homozygous Result



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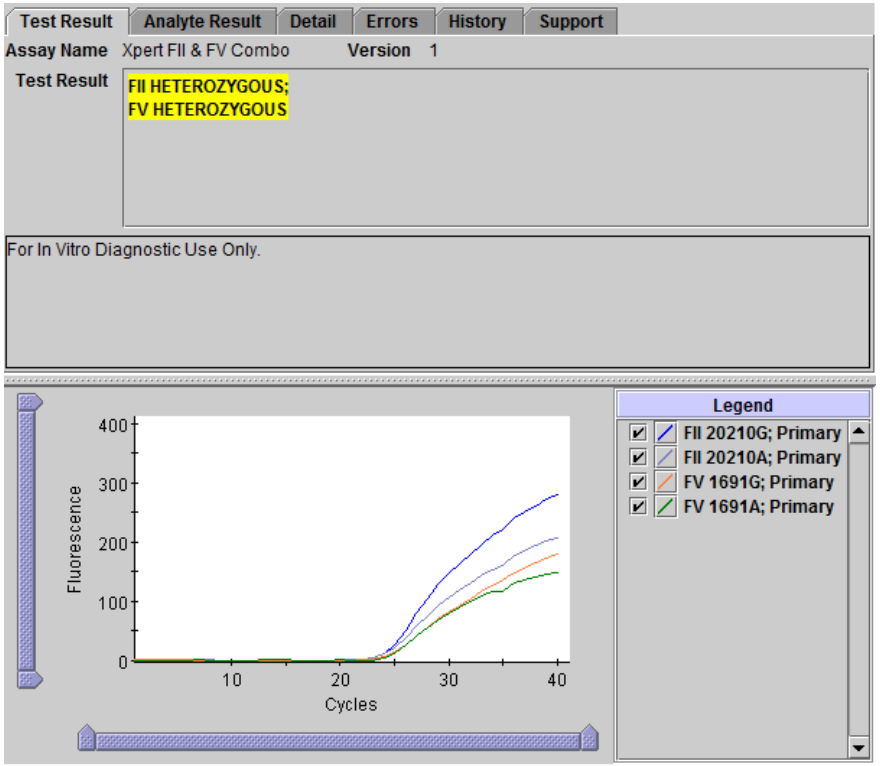
Xpert FII & FV Combo Heterozygous Result



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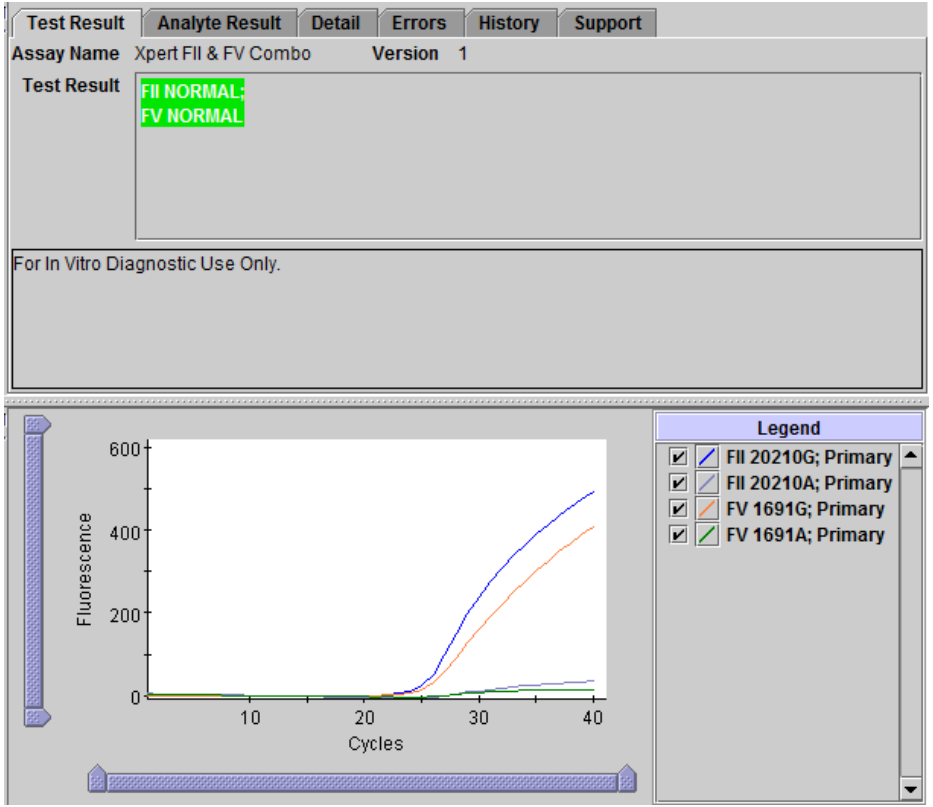
Xpert FII & FV Combo Normal Results



CE-IVD and US-IVD. For *in vitro* diagnostic use.

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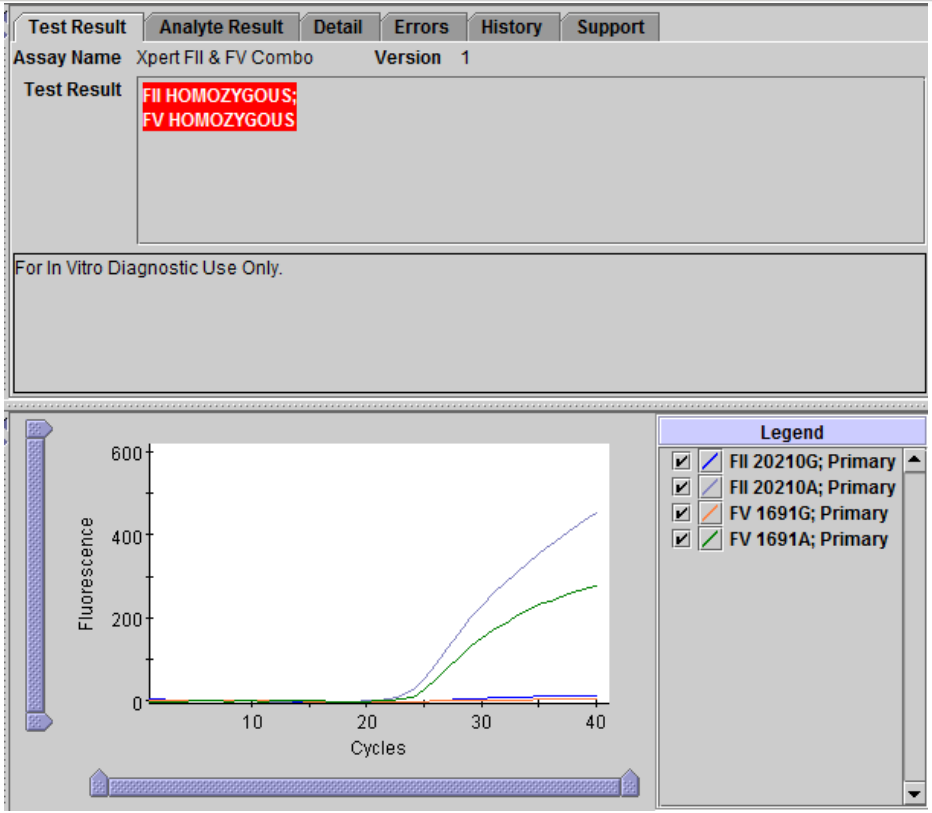
Xpert FII & FV Combo Homozygous Results



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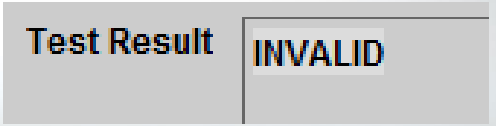
Reasons to Repeat the Assay

- An INVALID result indicates that the internal SPC failed. The sample was not properly processed or PCR was inhibited.

- An ERROR result indicates that the assay was aborted. Possible causes include: the reaction tube was filled improperly; a reagent probe integrity problem was detected; the maximum pressure limit was exceeded; a system component failed.

- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, a load error occurred, the software was closed prematurely, or a power failure occurred.

INVALID



Presence or absence of Factor II & Factor V normal and mutant alleles cannot be determined

- Probe Check—PASS; all probe check results pass.
- INVALID - presence or absence of Factor II/Factor V normal and mutant alleles cannot be determined:
  - Amplification did not happen
  - In the combinatorial test, one or no curve exists, result is invalid
  - In rare Factor V mutations (A1696G, G1689A, and A1692C)

ERROR

Presence or absence of Factor II & Factor V normal and mutant alleles cannot be determined

- ERROR
- Probe Check—FAIL*; one or more of the probe check results fail.
  - The Probe Check control failed and the assay aborted possibly due to an improperly filled reaction tube,
  - A probe integrity problem was detected.
  - Errors may also be caused by exceeding the maximum pressure limits
  - System component failure.

*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

- NO RESULT
- Probe Check—NA (not applicable)

- Insufficient data were collected to produce an assay result (for example, this can occur if the operator stopped a test that was in progress).

Xpert FII & FV Retest Procedure

1

Discard used cartridge.

Follow your institution's safety guidelines for disposal of cartridges.

2

Obtain the residual sample.

If the leftover sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample.

3

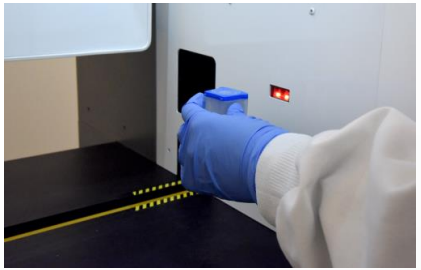
Obtain a new cartridge.

Label appropriately as retest on the new cartridge.

Process the sample per the package insert.

4

Run the test on the system.



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Factors That Can Negatively Affect Results

- 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.
- Incorrect storage of collected specimens can affect the result
  - Ensure that sample is well mixed and has not been through many thaw/freeze cycles.
  - Verify correct volume of blood was added to sample chamber
- 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.

Technical Support

- Cepheid provides technical support in the field, on the phone, by fax, and by email.

- Contact information for Cepheid offices is available at **http://www.cepheid.com/support**
  - Select the Contact Us option to access contact information
  - Complete online form to Create a Support Case

- Before contacting Cepheid Technical Support, collect the following information:
  Product name
  Lot number
  Serial number of the instrument
  Error messages (if any)
  Software version and, if applicable, Computer Service Tag Number


Thank You.
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