Assay Technical Training
Xpert® HCV VL Fingerstick

For CE-IVD Use Only

CE IVD In Vitro Diagnostic Medical Device

301-9713 Rev. B December 2018

Training Agenda

- **Xpert® HCV VL Fingerstick**
  - Clinical utility
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Preparing the cartridge
  - Quality Controls
  - Result analysis
- **Discussion**

Training Objectives

- **At the end of the training, user will be able to:**
  - Properly store and handle the Xpert® HCV VL Fingerstick cartridge kit
  - Follow proper laboratory safety precautions
  - Collect and transport appropriate specimen
  - Prepare a cartridge and run the assay
  - Report the various software generated results
  - Understand the assay control strategy

The Cepheid Solution

- Detects and quantifies
  - HCV RNA
  - Results within linear range 100 - 100,000,000 HCV RNA IU/mL*
- On-board controls for each sample
  - Sample Volume Adequacy (SVA)
  - Probe Check Control (PCC)
  - Internal Quantitative Standards (IQS) High (H) and Low (L)
- Results in approximately 60 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

* The assay is standardized against the 4th World Health Organization (WHO) International Standard for HCV (NIBSC code: 06/100).6

Intended Use

The Xpert HCV VL Fingerstick (FS) assay is an *in vitro* reverse transcription polymerase chain reaction (RT-PCR) assay for the detection and quantification of Hepatitis C Virus (HCV) RNA in human capillary fingerstick EDTA whole blood and venous EDTA whole blood from HCV-infected individuals using the automated GeneXpert® Instrument Systems.

The Xpert HCV VL Fingerstick assay is intended for use as an aid in the initial diagnosis in individuals at high risk of HCV infection or in anti-HCV positive individuals. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.

The Xpert HCV VL Fingerstick assay is intended for use as an aid in the management of HCV infected patients undergoing antiviral therapy. The test measures HCV RNA levels at any time during viremia and during treatment; and can be utilized to predict sustained and nonsustained virological responses to HCV therapy.

Refer to Package insert for full detail

Intended Use - Remarks

- The Xpert HCV VL Fingerstick assay is intended to be used by **laboratory professionals or specifically-trained healthcare workers.**
- The assay is **not** intended to be used as a blood donor screening test for HCV.
- The assay can quantify HCV RNA over the range of **100 - 100,000,000 IU/mL**

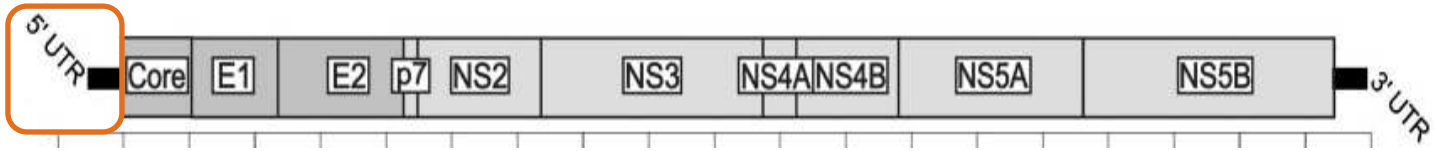
CE IVD In Vitro Diagnostic Medical Device

Targets

Targets

- Internal Quantitative Standards (IQS-H) and (IQS-L)
- Most conserved region of the HCV RNA genome

The assay is designed to detect 6 genotypes (1-6)



Assay Requirements

GeneXpert Systems

- GeneXpert Dx Software **v 4.7 b** or higher
- Xpertise Software **v 6.4** or higher

Test Kits (CE-IVD)

- GXHCV-FS-CE-10

Sample Collection

- Disposable Safety-Lancet Super, 1.5 mm (Sarstedt, P/N: 85.1018) or similar, to draw a minimum of 100µL capillary blood- Not provided
- Minivette® POCT 100 µL K3 EDTA for capillary blood collection – Can be ordered from Cepheid
- EDTA tubes for venous blood venipuncture – Not provided

Other materials

- Personal Protective Equipment (PPE)
- 1:10 bleach
- 70% ethanol or denatured ethanol
- Disinfecting wipes

Optional

- Uninterruptible Power Supply /Surge Protector
- Printer

Good Laboratory Practice

Personnel Protective Equipment (PPE)	• Wear clean lab coats and gloves • Change gloves between processing samples
Lab Bench area	• Clean work surfaces routinely with: ✓ 1:10 dilution of household bleach ✓ 70% Ethanol Solution • After cleaning, ensure work surfaces are dry
Specimens, Samples, and Kits Storage	• Store specimens and sample away from kit to prevent contamination
Equipment(s)	• Use filtered pipette tips when recommended • Follow the manufacturer's requirements for calibration and maintenance of equipment(s)

Kit Handling

Xpert HCV Viral Load Fingerstick Kit Contents

Catalog Number	GXHCV-FS-CE-10
Cartridges Per Kit	10
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Storage	2-28 ˚C

Kit of 10 cartridges

Cartridges contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.

Xpert HCV VL Fingerstick Kit Storage and Handling

- Store the Xpert HCV VL Fingerstick cartridges at 2-28˚C
- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use Collection devices that have not been validated by Cepheid
- Open the assay cartridge lid only when adding the Sample, close the lid and proceed with processing
- Start the test within 4 hours of adding the sample to the cartridge

Warnings and Precautions

- Do not shake the cartridge
- Do not use a cartridge that… :
  - appears wet, has leaked or if the lid seal appears to have been broken
  - appears damaged
  - has been dropped after removing it from packaging
  - has been dropped or shaken after adding the sample to it
  - has a damaged reaction tube
  - has been used: each cartridge is single-use to process one test
  - is expired
- Do not reuse spent disposable pipettes

Dispose Xpert HCV VL Fingerstick Assay cartridges and reagents according to your institution's and country's guidelines for disposal of hazardous materials

Warnings and Precautions

- Biological specimens, collection 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

Specimen Collection, Storage and Transport

Specimen Collection – Capillary Blood

- Recommended puncture device (not provided by Cepheid)
  - Disposable Safety-Lancet Super,1.5 mm
  - Reference Sarstedt, P/N: 85.1018 or similar

- Mandatory Collection device (Available with Cepheid)
  - Minivette® POCT (100µL)

Picture: Sarstedt brochure

- Process:
  - Warm up the finger
  - Sterilize the area before collecting the sample
  - Prick the finger with Safety Lancet and collect blood following manufacturer‘s instruction

Detailed instructions in the next slides

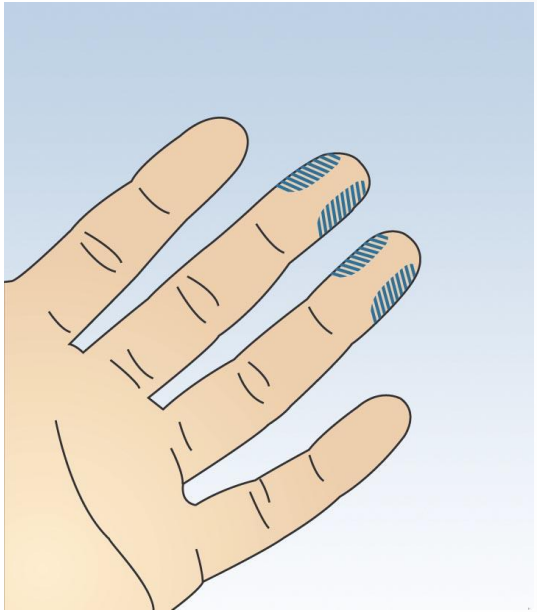
Manufacturer’s instructions

Warming the puncture site enhances blood flow by **up to 7 times** the normal value

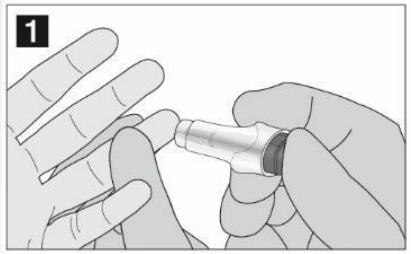
- Wrap the hand of the patient in a warm cloth (39˚C - 40˚C)

or

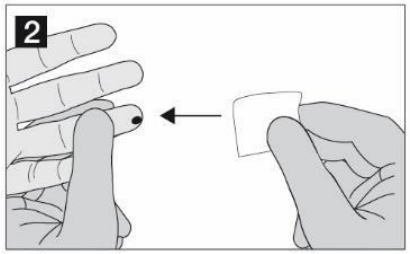
- Hold the patient’s hand under 39 to 40 ˚C running water.



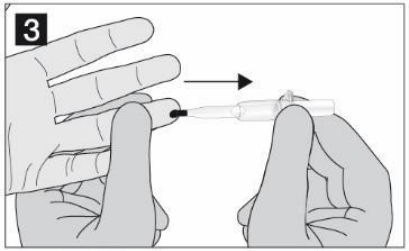
Capillary Blood Collection



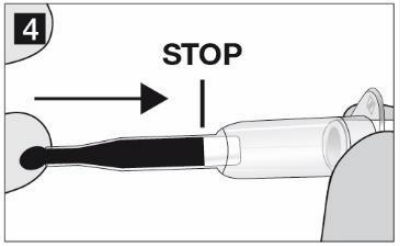
Clean the puncture site using a disinfectant
Leave to dry until the disinfectant has completely dried
Prick the finger with Safety Lancet



Wipe off the first drop
Hold the punctured site horizontally
Avoid smearing the blood drop



Touch the blood drop with the tip of the Minivette® POCT and hold it in a horizontal position



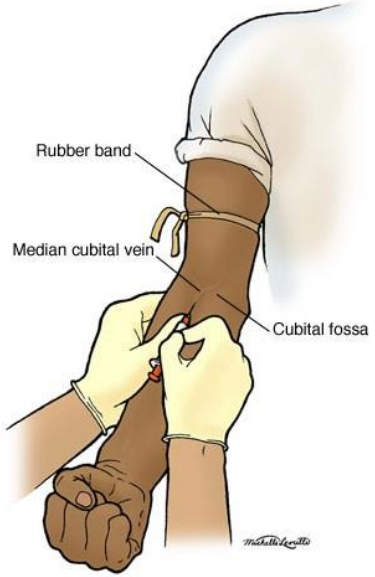
Wait until the blood has reached the filter at the other end of capillary tip

The air ventilation hole at the end of the piston should not be covered, nor pushed down.

Video : https://www.sarstedt.com/en/products/new-products/video-minivetter-poct/

Specimen Collection – Venous Blood

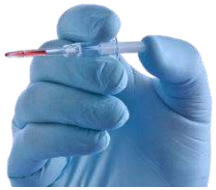
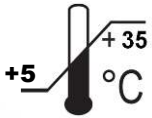
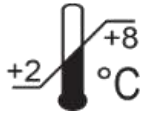
- Collect venous blood according to EDTA tube manufacturer's instructions
- Immediately invert the tube 8-10 times to mix to ensure adequate anticoagulation of the specimen



Medicoinfo.in

Specimen Transport and Storage

Picture: MarketLab.com

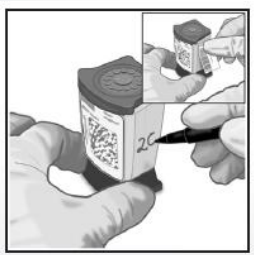
Sample	Temperature (˚C)	Storage Time
 Capillary blood EDTA		Maximum 15 min from collection
 Venous blood EDTA		72 hours
		24 hours

Cartridge Preparation

Cartridge Preparation – Capillary blood from Minivette

NOTE:

- Collect 100µL of the whole blood sample using the Minivette
- Allow the cartridge to adjust to room temperature prior to starting the test



1. Label the side of the cartridge with the Sample ID.

2. Open the cartridge lid.

3. Place the Minivette all the way in the sample chamber of the cartridge and slowly press down the piston to dispense the whole blood sample.

4. Close the cartridge lid.

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

Cartridge Preparation – Venous Blood in EDTA Tube

NOTE:
- Collect venipuncture blood (EDTA) per manufacturer's instructions
- Allow the cartridge to adjust to room temperature prior to starting the test

1. Label the side of the cartridge with the Sample ID.

2. Open the cartridge lid.

3. Mix the venous blood contained in EDTA collection tube by inverting the tube at least 7 times.

4. Pipette 100 µL of venous blood from the collection tube with an automatic micropipette. You may use the pre-wetting to avoid pipetting mistakes

5. Place the pipette in the sample chamber and slowly transfer 100µL of venous blood from the collection tube into the sample chamber of the cartridge.

6. Close the cartridge lid.

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

Run a Test

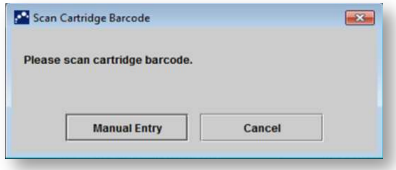
1 Create Test

GeneXpert

Start the test within **4 hours** after adding the sample to the cartridge

2 Scan barcode messages:

Cartridge/ Patient and/or Sample ID

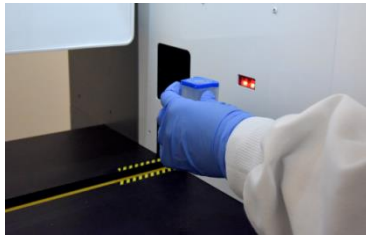


By default, do not click on **Manual Entry** or **Cancel**

3 Scan the cartridge

GeneXpert Infinity

Place the cartridge on the conveyor after adding the sample to the cartridge



"For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx or Xpertise Dx Operator Manuals.

Create a Test on GeneXpert Dx Software

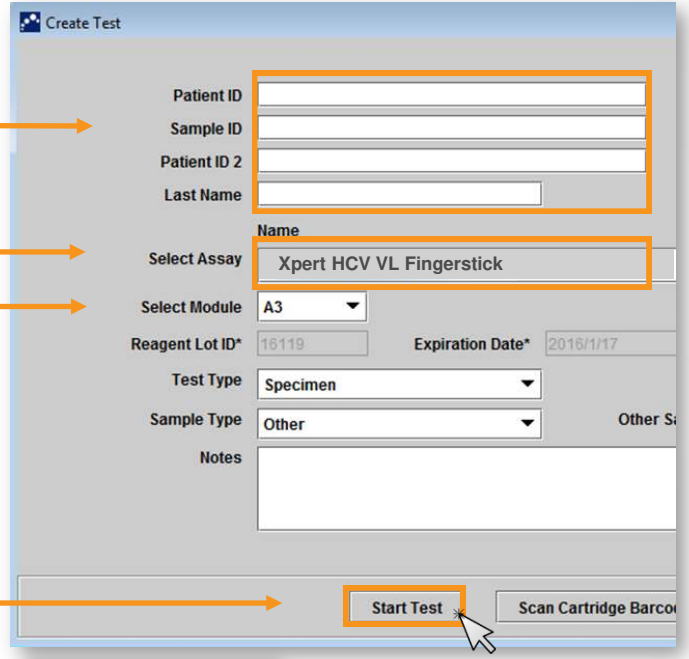
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 The module is selected automatically
DO NOT CHANGE IT!!!

7 Click on Start Test

8 A green light will flash on the module
Load the cartridge into module and close the door



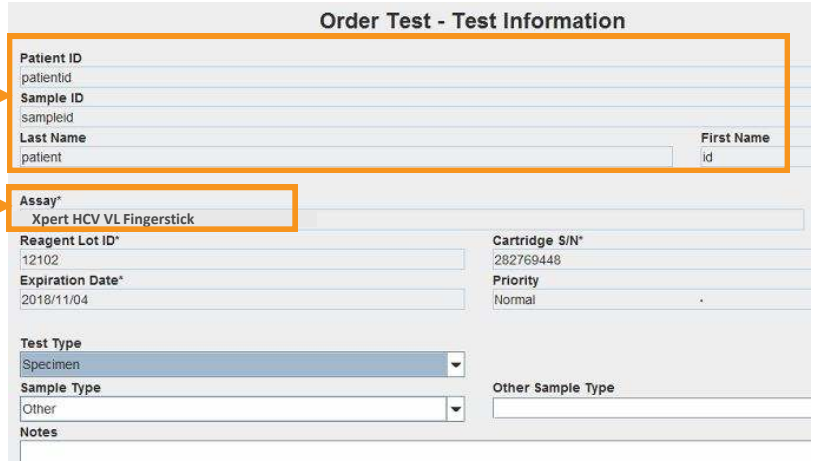
Create a Test on Xpertise Dx Software

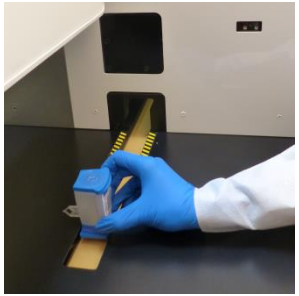
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 Click on SUBMIT

7 Place the cartridge into the conveyor belt





Automated Xpert Protocol

1 Sample is added to the cartridge

2 The cartridge is loaded into the instrument

3 Nucleic acids are purified

4 Purified Nucleic acids mix with the PCR reagents

5 Simultaneous amplification and detection occurs

6 Results are ready to view

Cepheid.

Quality Controls

Cepheid Control Strategy

CONTROL

- **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.

- **Assay Quality Controls**
  - 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
    - Sample Volume Adequacy (SVA)
    - Probe Check Controls (PCC)
    - Internal Quantitative Standards IQS-L and IQS-H to quantify the HCV RNA virus

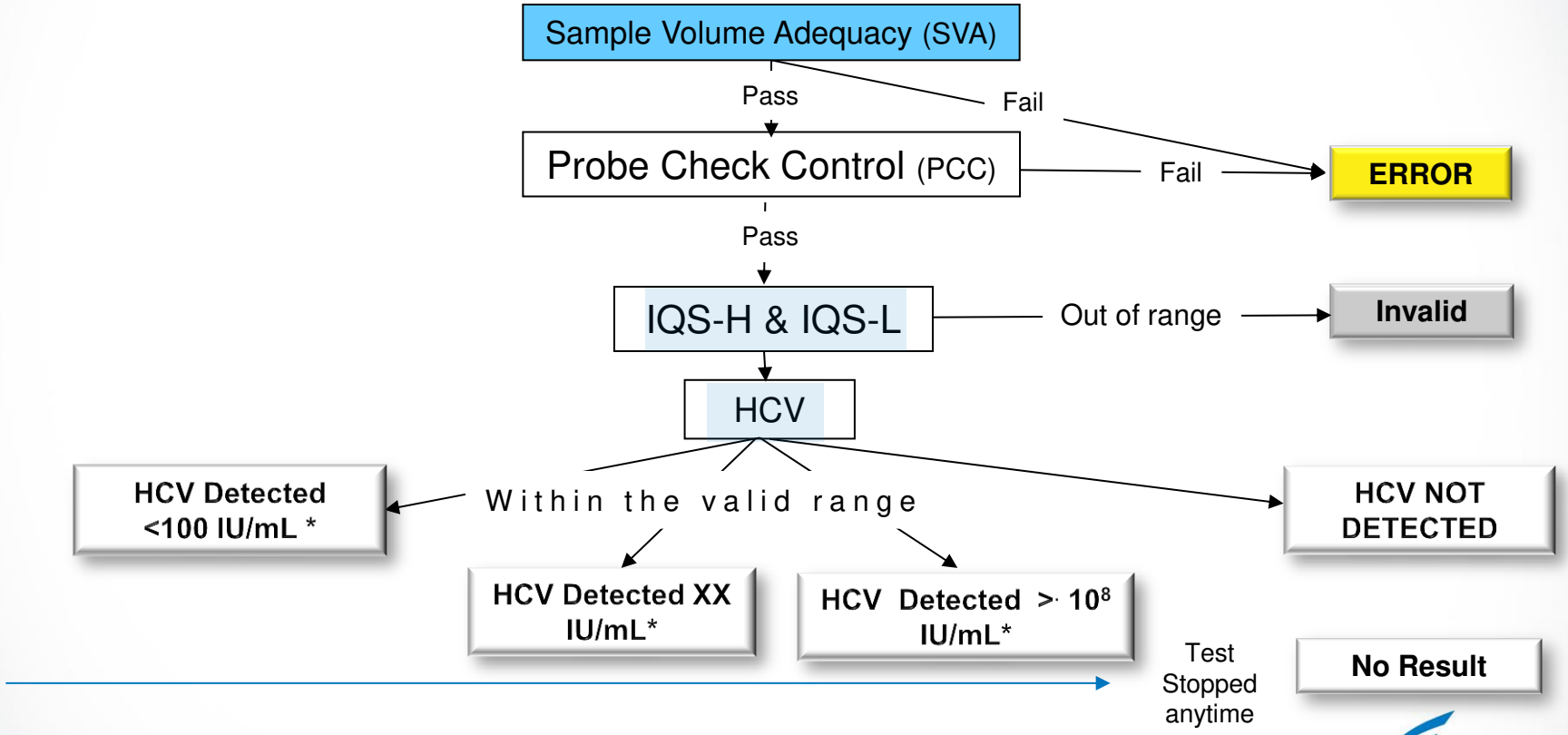
Internal Quality Controls in details

- **Sample Volume Adequacy (SVA)**

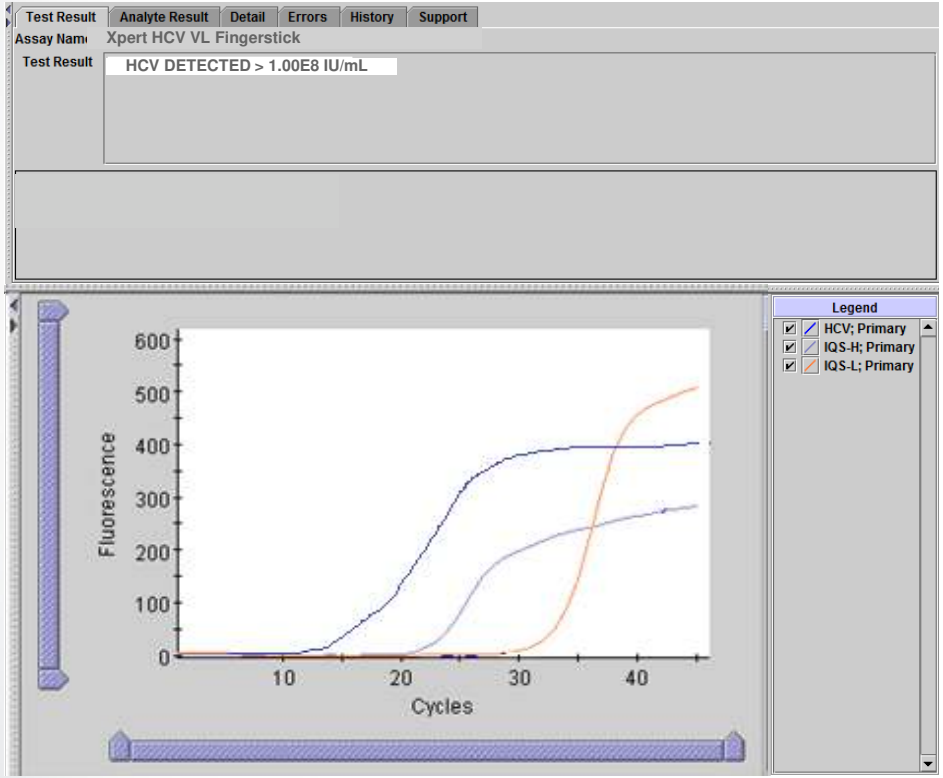
  Verifies that the correct sample volume is added to the cartridge
- **Probe Check Controls (PCC)**
  - Before the PCR step, the fluorescence signal is measured from all probes and compared with default settings to monitor
    - bead rehydration
    - probe integrity
    - reaction tube filling
    - dye stability
- **Internal Quantitative Standard (IQS)**
  - 2 non infectious armored RNA® controls non specific to HCV in the form of a dry bead
  - Called IQS Low (10^3 copies /mL) and IQS High (10^6 copies/mL)
  - The IQS-H and IQS-L are calibrated against the 4th WHO International standard for HCV NAT
  - Verifies that sample was correctly processed
  - Verifies lysis, presence of the organism and detects inhibition
  - IQS Low and IQS High Ct and fluorescence values must always be within the valid range

Result Interpretation

Result Interpretation Algorithm



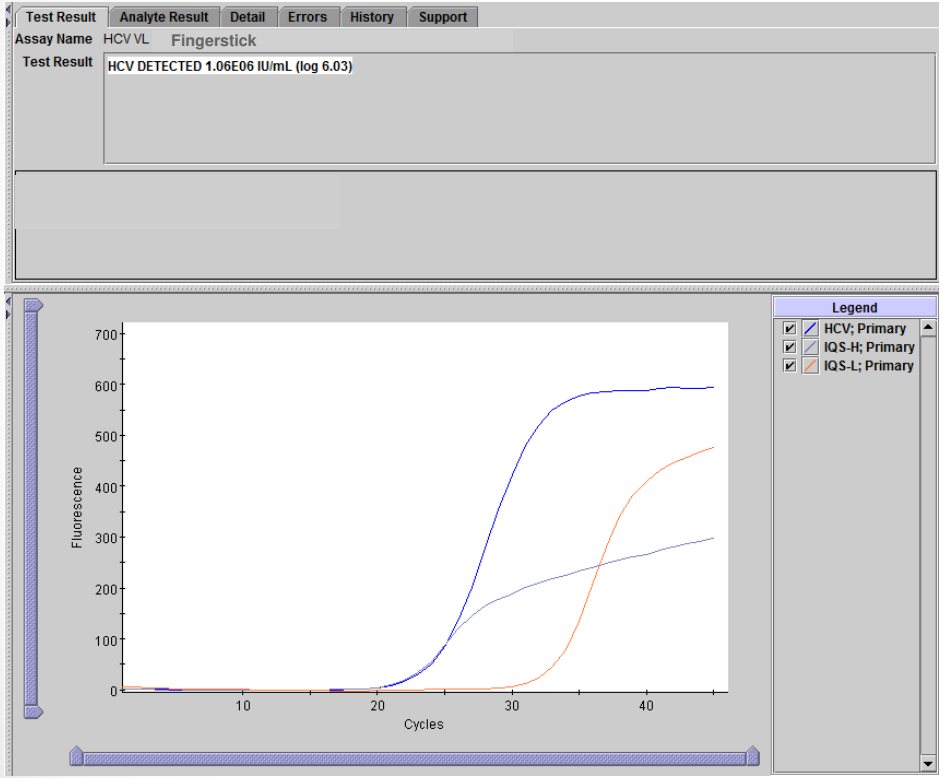
HCV DETECTED > 1.00E08 IU/mL



The target HCV VL is detected above the analytical measurement range

- IQS-H: PASS
  - IQS-H has a Ct value within the valid range
- IQS-L: PASS
  - IQS-L has a Ct value within the valid range
- Probe Check: PASS

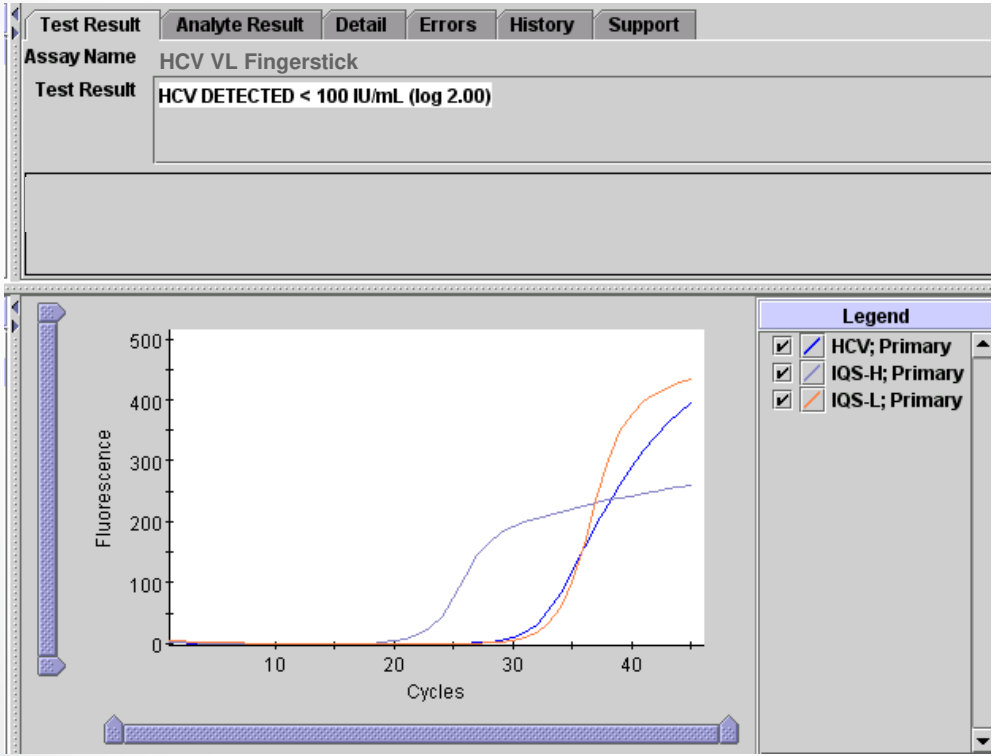
HCV DETECTED 1.06 x 10^6 IU/mL



HCV RNA has a quantitative value within the analytical measurement range

- IQS-L and IQS-H — PASS
- Probe Check — PASS; all probe check results pass

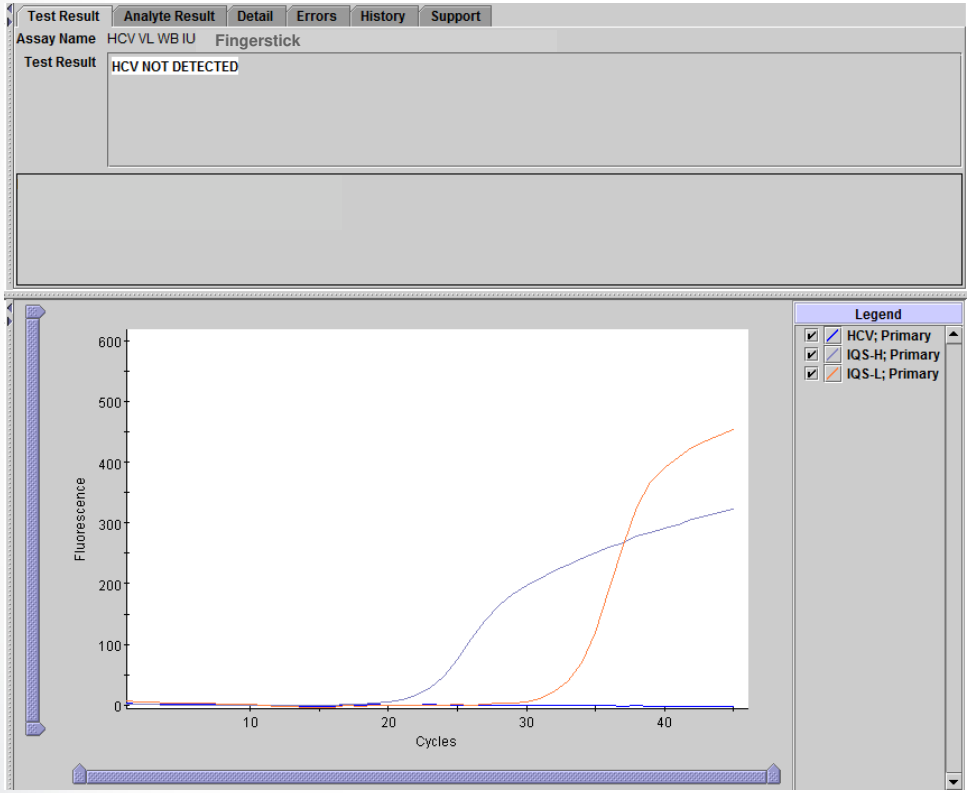
HCV VL DETECTED < 100 IU/mL



The target HCV VL is detected below the analytical measurement range

- IQS-H: PASS
  - IQS-H has a Ct value within the valid range
- IQS-L: PASS
  - IQS-L has a Ct value within the valid range
- Probe Check: PASS

HCV NOT DETECTED



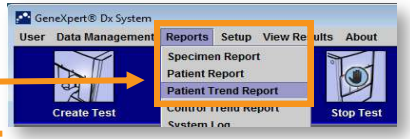
The HCV RNA target is not detected

- IQS-L and IQS-H — PASS
- Probe Check — PASS; all probe check results pass

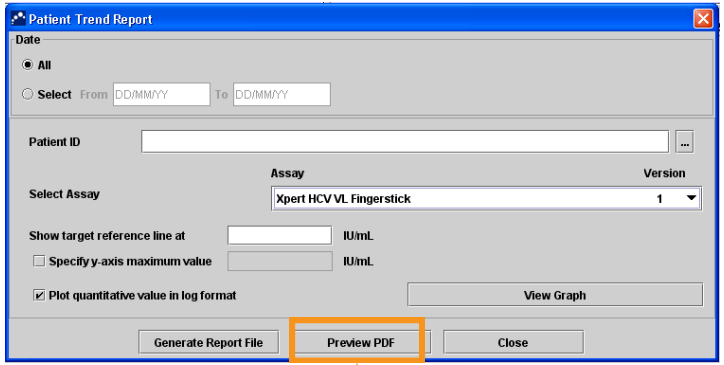
Patient Trend Report

- **You can monitor a specific patient over a period of time by creating a trend report**

1. Select Reports and Patient Trend Report

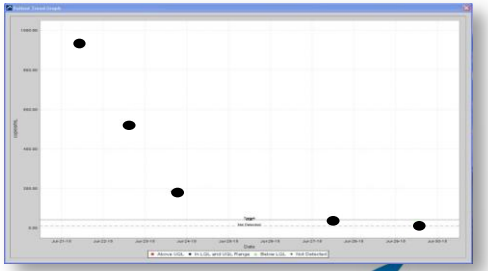


2. Choose
   - Time range
   - Patient ID
   - Assay
   - Target preferences



4. Preview the Report

5. View your Report



Available only in Software version GxDx 4.6a and higher

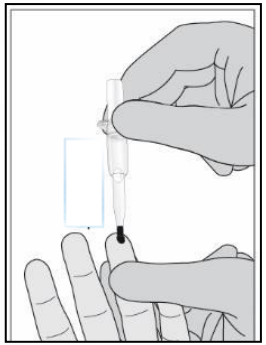
Troubleshooting

Factors That Negatively Affect Results

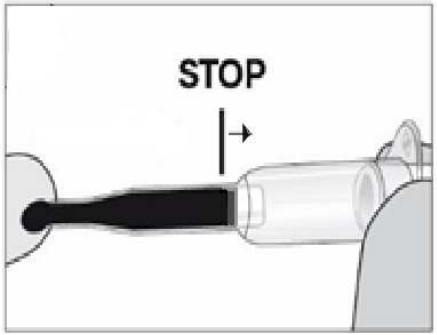
- Improper specimen collection
  - Performance with other collection devices and specimen types has not been assessed
- Improper transport or storage of collected specimen
  - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
  - Modification to the testing procedures, technical error and sample mix-up may impact the test results
  - Careful compliance with the package insert is necessary to avoid erroneous results
- Interfering substance
  - False negative results or invalid results may be observed in the presence of interfering substance

Incorrect Sample Collection

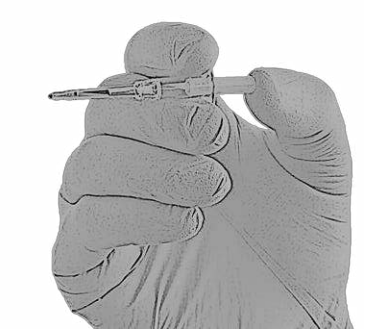
Do not place the Minivette vertically



Do not aspirate into filter



Do not block the piston while collecting



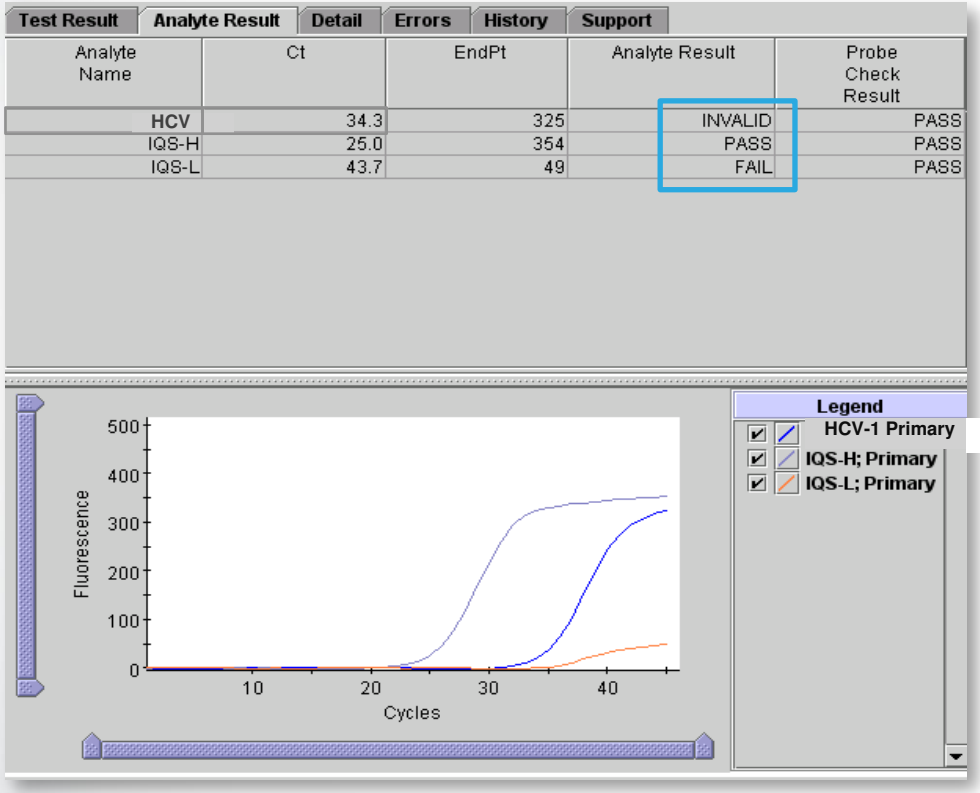
Do not store Minivette > 15 min.

Do not touch the finger
(only touch the blood drop)

Cepheid

INVALID Result

INVALID



Presence or absence of the HCV target can not be determined

- IQS-H and or IQS-L: FAIL

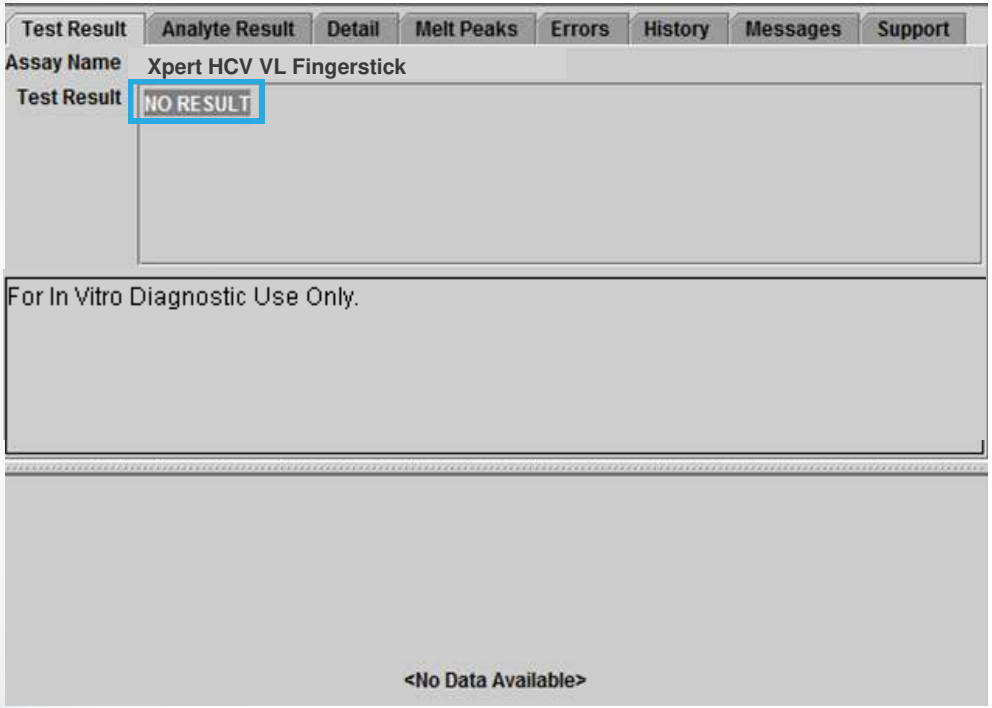
  Internal Quantitative Control Cycle thresholds are not within the valid range

- Probe Check: PASS

– Cause
  – Improper sample collection (using heparin tube for e.g)
  – Incorrect sample preparation
  – Improper storage of the cartridges
  – Inefficient sample processing in cartridge
  – Missing primer/probe or enzyme beads
  – Presence of inhibitors in the sample
– Solution
  – Repeat the test with a new cartridge and new sample

NO RESULT

NO RESULT



- The presence or absence of HCV cannot be determined.
- A NO RESULT indicates that insufficient data were collected.
- IQS-H or IQS-L: NO RESULT
- Probe Check: NA (not applicable)
- **Cause**
  - Test was stopped with stop test button
  - Electrical failure
- **Solution**
  - Secure the power
  - Repeat the test with a new cartridge

Re-test Procedure

1

Discard used cartridge

Follow your institution's safety guidelines for disposal of cartridges

2

Obtain the residual sample. Mix as per Package Insert

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

3

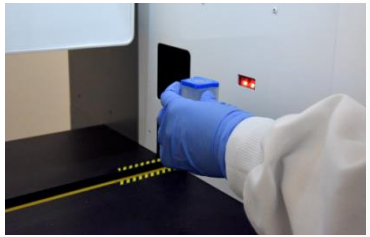
Obtain a new cartridge

Label appropriately as retest on the new cartridge

Process the sample per the package insert

4

Run the test on the GeneXpert System



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Technical Assistance

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

- Log your complaint online using the following link ***http://www.cepheid.com/us/support*** **:** *Create a Support Case*

Region	Telephone	Technical Support Email
US	+ 1 888 838 3222	techsupport@cepheid.com
Australia and New Zealand	+ 1800 130 821 (AU) + 0800 001 028 (NZ)	techsupportANZ@cepheid.com
Brazil and Latin America	+ 55 11 3524 8373	latamsupport@cepheid.com
China	+ 86 021 5406 5387	techsupportchina@cepheid.com
France	+ 33 563 825 319	support@cepheideurope.com
Germany	+ 49 69 710 480 480	support@cepheideurope.com
India, Bangladesh, Bhutan, Nepal, and Sri Lanka	+ 91 11 48353010	techsupportindia@cepheid.com
Italy	+ 39 800 902 567	support@cepheideurope.com
South Africa	+ 27 861 22 76 35	support@cepheideurope.com
United Kingdom	+ 44 3303 332 533	support@cepheideurope.com
Belgium and Netherlands	+33 563 825 3319	support@cepheideurope.com
Other European, Middle East, and African countries	+ 33 563 825 319 + 971 4 253 3218	support@cepheideurope.com


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