Assay Technical Training
Xpert® HCV Viral Load

For CE-IVD Use Only

Cepheid Training Center

CE IVD In Vitro Diagnostic Medical Device

301-4915 Rev. B August 2018

Training Agenda

- **Xpert® HCV Viral Load**
  - Clinical utility
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Preparing the cartridge
  - Quality Controls
  - Results analysis
- **Discussion**

Training Objectives

- **At the end of the training, user will be able to:**
  - Properly store and handle the Xpert® HCV Viral Load cartridge kit and sample collection kits
  - 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 target
  - HCV RNA
  - Results within linear range of 10 to 100,000,000 IU/mL
- On-board internal controls for each sample
  - Sample Volume Adequacy (SVA)
  - Probe Check Control (PCC)
  - Internal Quantitation Standards IQS-H and IQS-L
- Results in approximately
  - 105 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results 24/7
- Random access

Intended Use

- The Xpert® HCV VL assay, performed on GeneXpert® Instrument Systems, is designed for the rapid quantitation of Hepatitis C Virus (HCV) RNA in human serum or plasma (EDTA) from HCV-infected individuals. The test utilizes automated reverse transcriptase polymerase chain reaction (RT-PCR) using fluorescence to detect the RNA of interest for the quantitation of HCV.

- The Xpert HCV VL assay quantifies HCV genotypes 1–6 over the range of 10 to 100,000,000 IU/mL.

- The Xpert HCV VL 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 baseline and during treatment and can be utilized to predict sustained and non-sustained virological response to HCV therapy.

Intended Use - Remarks

- Results from the Xpert HCV VL assay may also be used to confirm HCV infection in anti-HCV positive individuals. In anti-HCV positive individuals who test negative for HCV RNA, use of another HCV antibody assay may be considered for distinction between true HCV exposure and biologic false positivity.

- Repeat HCV RNA testing may be indicated in cases that have had HCV exposure in the last 6 months or have clinical evidence of HCV disease.

- The assay is not intended to be used as a donor screening test for HCV.

Targets and probes

Targets

– Internal Quantitative Standards (IQS-H) and (IQS-L)

– Most conserved region of the HCV RNA genome

Probes

- 3 probes (HCV, IQS-H and IQS-L)

Xpert® HCV Viral Load Requirements

GeneXpert Systems

- GeneXpert Dx Software **V4.7b** or higher
- Xpertise Software **V6.4b** or higher

Test Kits (CE-IVD)

- GXHCV-VL-CE-10

Sample Collection

- Whole blood collected in K2-EDTA, EDTA-PPT, or serum collection tubes

Other materials

- Personal Protective Equipment (PPE)
- 1:10 Bleach
- 70% ethanol or denatured ethanol
- Vortex Mixer
- Centrifuge for plasma preparation

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 tips when recommended • Follow the manufacturer's requirements for calibration and maintenance of equipment(s)

Kit Handling

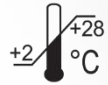
Xpert® HCV Viral Load Kit Contents

Catalog Number	GXHCV-VL-CE-10
Cartridges Per Kit	10
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Transfer Pipettes	10 (1mL)
Storage	2-28 ˚C

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

Xpert® HCV Viral Load Kit Storage and Handling

- Store the HCV VL Assay cartridges and reagents at 2–28˚C



- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use Collection Reagent tubes that have not been validated by Cepheid
- Open the Assay cartridge lid only when adding the Sample, close the lid and proceed with processing

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 Assay cartridges and reagents according to your institution's and country's guidelines for disposal of hazardous materials

Warnings and Precautions

- 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.

Xpert® HCV Viral Load Limitations

- This assay is not intended to be used as a donor screening test
- The performance of Xpert HCV VL has not been demonstrated from other specimens than the ones validated, i.e plasma, serum
- The performance of the Xpert HCV VL test has not been evaluated with specimens processed by methods other than those described in the package insert
- Failure to follow assay procedures may lead to false results
- Inhibitors present in the samples may lead to invalid results

For detailed information, refer to the current Package Insert

Specimen Collection, Storage and Transport

Xpert® HCV Viral Load Collection

- **Whole blood**

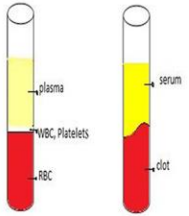
➢ Collect whole blood specimens in EDTA, EDTA-PPT or Serum tubes per manufacturer's instructions

EDTA tube

Serum tube

Heparin tube

- **Plasma/serum**
  - Centrifuge to separate the plasma/serum and red blood cells per the manufacturer's instructions
  - Prepare minimum of 1.2 mL of plasma/serum



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Specimen Collection, Transport and Storage

	Prior to testing	Temperature (˚C)	Storage Time
	**EDTA anticoagulated whole blood**	+15 +35 °C	24 hours
		+2 +8 °C	72 hours

Plasma specimens are stable up to three freeze/thaw cycles.

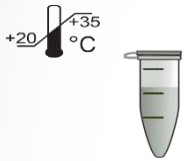
	Prior to testing	Temperature (˚C)	Storage Time
 	**Plasma and Serum**	+15 +35 °C	24 hours
		+2 +8 °C	3 days
		-70 -18 °C	6 weeks

Cartridge Preparation

Xpert® HCV VL Cartridge Preparation

Specimen: Plasma / Serum

1

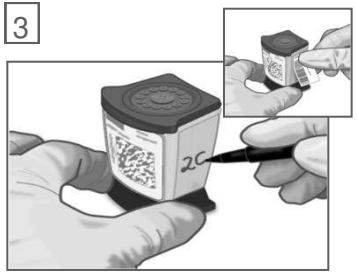


If using frozen or refrigerated samples, place at room temperature until completely thawed and equilibrated to room temperature

2

Vortex the equilibrated sample for 15 seconds. If specimen is cloudy, centrifuge it for a few seconds

3

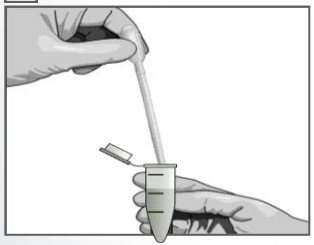


Label the side of the cartridge with the sample ID as the

4

Open the cartridge lid

5



Pipette at least 1 mL of the sample using the provided pipette*

6

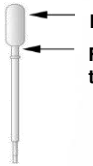
Slowly empty the pipette into the sample chamber of the cartridge

7

Close the lid firmly.
Start the test within the time frame specified in the package insert.

8

Start the test on your GeneXpert Instrument



HCV VL Assay Transfer pipette

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Run a Test

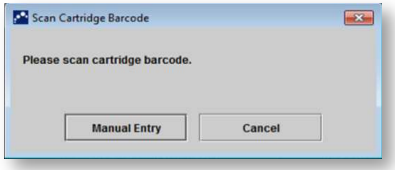
1 Create Test

GeneXpert

GeneXpert Infinity

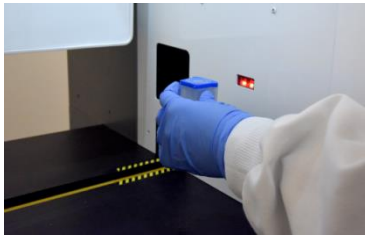
2 Scan barcode messages:

Cartridge/ Patient and/or Sample ID



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

3 Scan 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

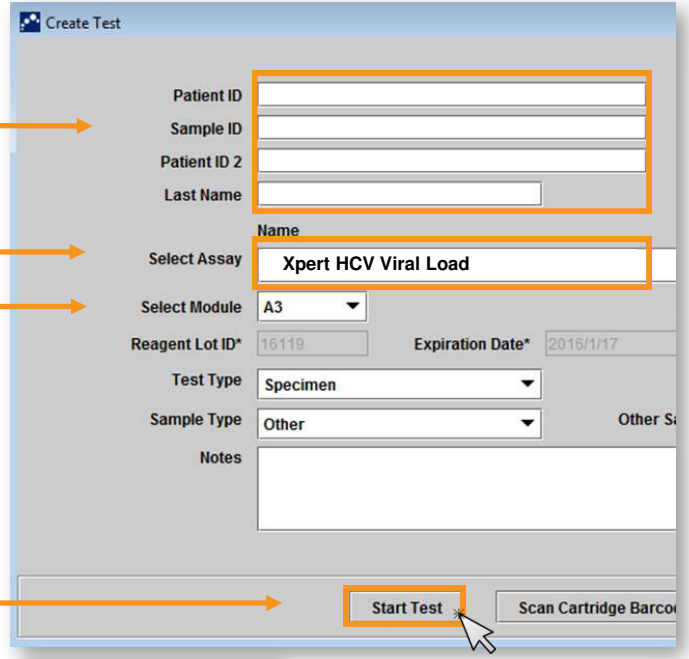
4 Complete the fields as required

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

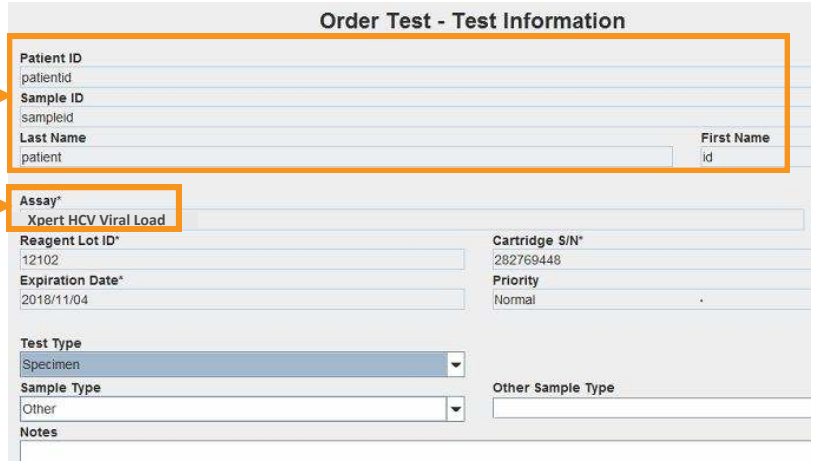


Create a Test on Xpertise Dx Software

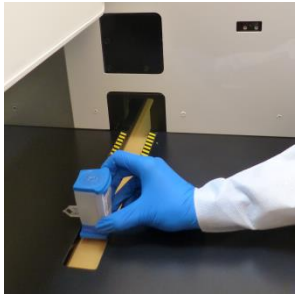
4 Complete the fields as required

5 The Assay Name Protocol is selected automatically

6 Click on SUBMIT



7 Place the cartridge into the conveyor belt



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Automated Xpert Protocol

1. Sample is added to the cartridge
2. The cartridge is loaded into the System
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

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Quality Controls

Assay Control Strategy

CONTROL

- **Xpert HCV VL 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

- **Sample Volume Adequacy (SVA)**
  - Ensures that the correct sample volume was added to the cartridge
- **Probe Check Controls (PCC)**
  - Before the PCR step fluorescent signal is measured on all probes and compared with pre-established factory settings to monitor
    - bead rehydration
    - reaction tube filling
    - probe integrity
    - dye stability
- **Internal Quantitative Standard High and Low (IQS-H and IQS-L)**
  - Called IQS Low (10^3 copies /ml) and IQS High (10^6 copies/ml)
  - 2 noninfectious armored RNAs® constructs
  - Ensures the sample was correctly processed
  - Detect specimen-associated inhibition of the RT-PCR reaction and presence of organism
  - Ct and fluorescence values must always be within the valid range

Commercially Available External Controls

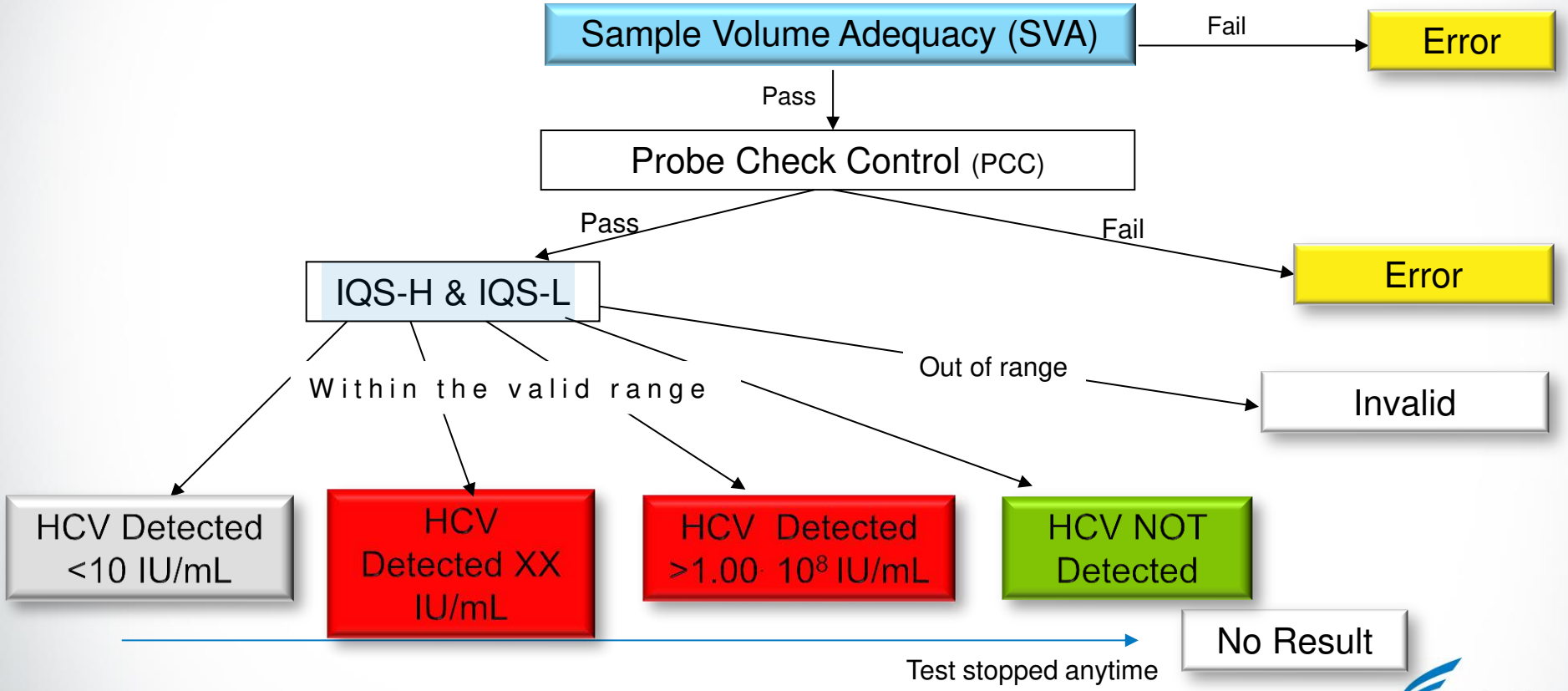
Thermofisher - https://www.lifetechnologies.com

Part Number	Description	Configuration	Storage
963003	HCV Positive High Control	1.2 mL	- 70 ˚C
963002	HCV Positive Mid Control	1.2 mL	- 70 ˚C

- Many other vendors for quality control material are also available than the one outlined above.
- External controls should be used in accordance with local, state accrediting organizations, as applicable.

Result Interpretation

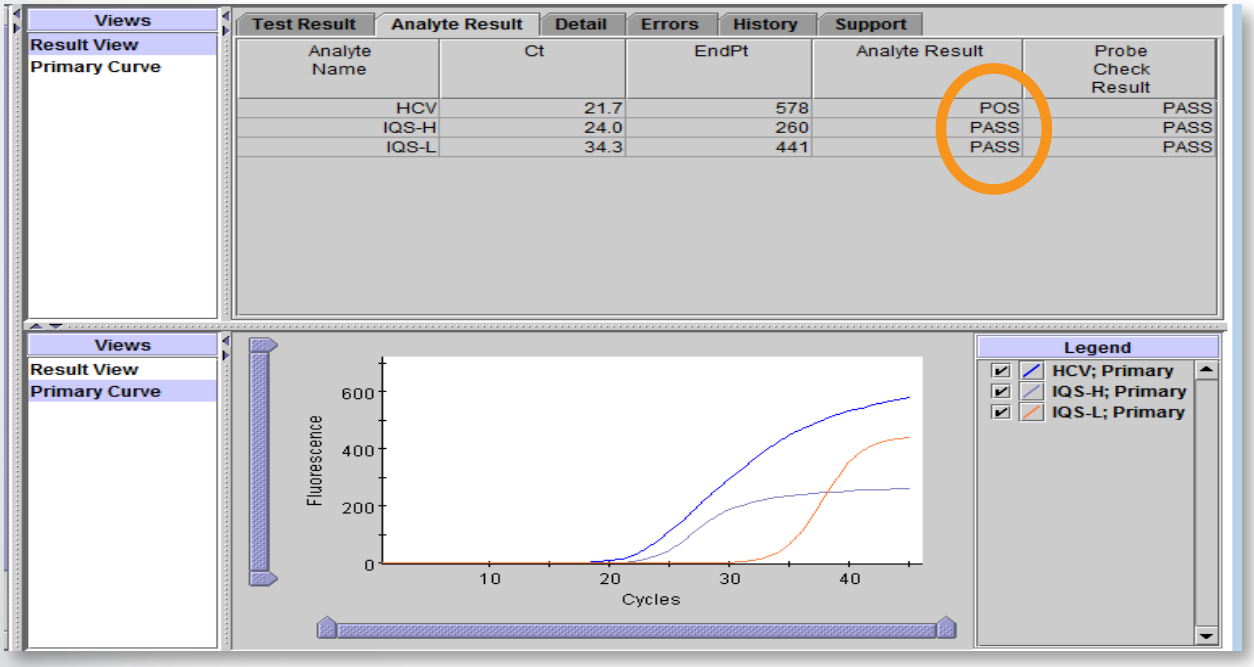
Result Interpretation Algorithm



HCV DETECTED xx IU/mL

Test Result	HCV DETECTED

Test Result	HCV DETECTED 3.02E05 IU/mL (log 5.48)



- The target HCV is detected at a quantitative value
  - The HCV RNA has a titer within the linear range setting of the assay and the endpoint above the minimum
- 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

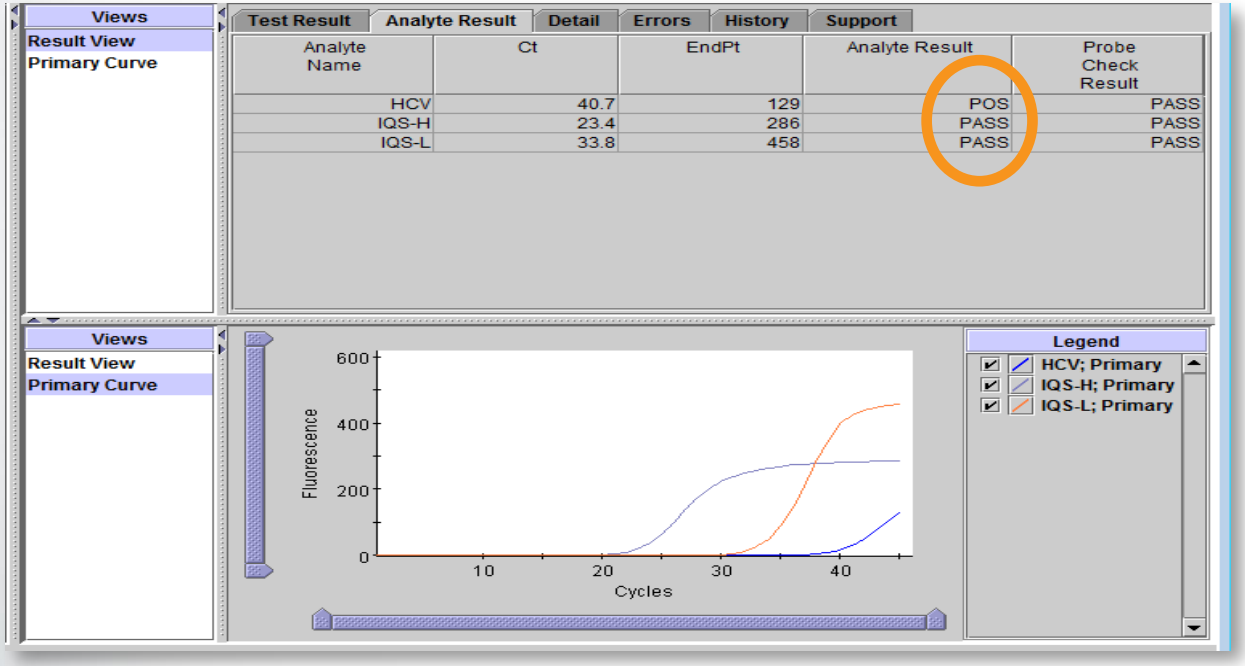
Example calculation:

3.02E05 = 3.02 x 10^5 = 302 000 IU/mL

HCV DETECTED < 10 IU/mL

Test Result	HCV DETECTED

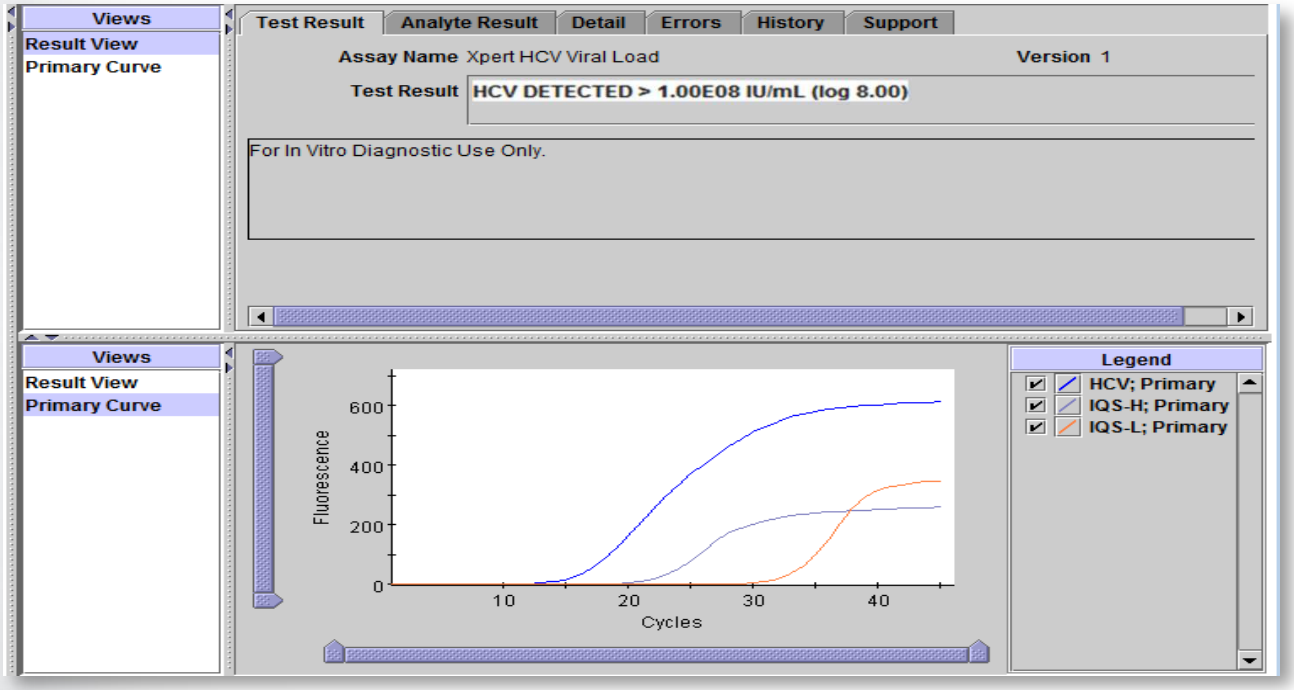
Test Result	HCV DETECTED < 10 IU/mL (log 1.00)



- The target HCV is detected below the quantitative range of the assay
- 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 x 10^8 IU/mL

Test Result	HCV DETECTED



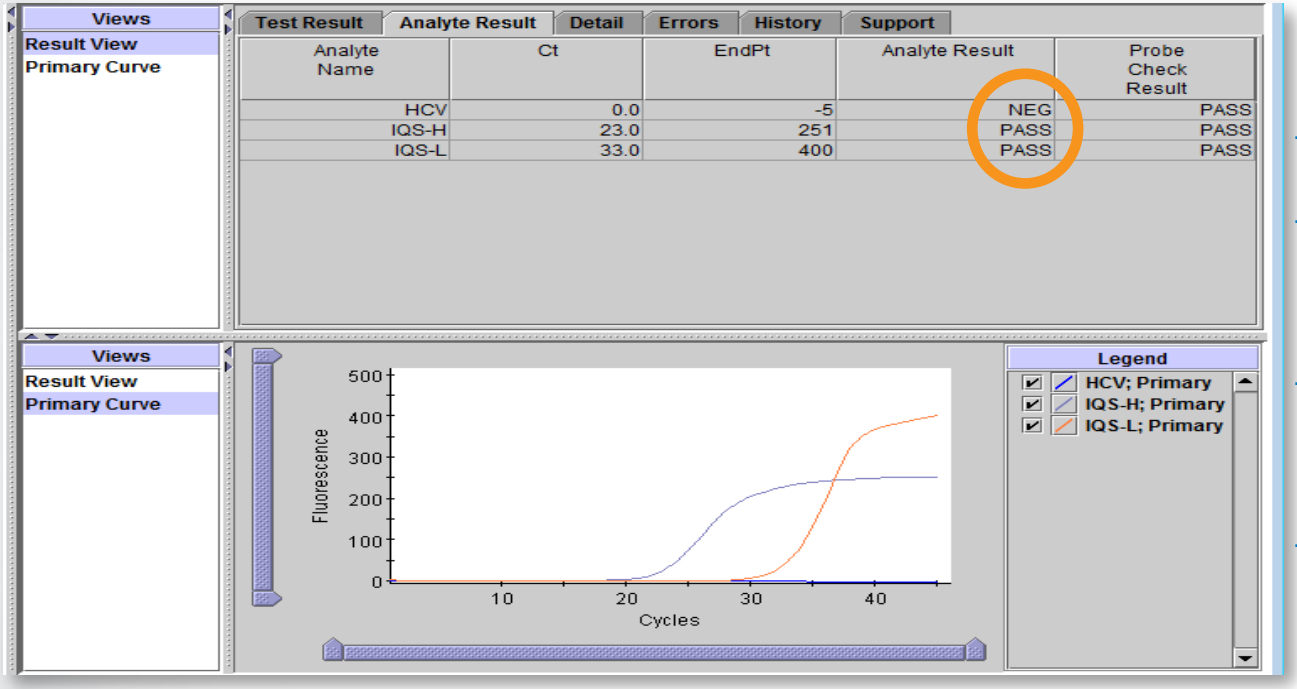
- The target HCV is detected above the quantitative range of the assay
- IQS-H: PASS
  - IQS-H has a Ct value within the valid range
- IQS-L: PASS
  - IQS-L has a Ct value within the valid range
- Probe Check: PASS

Example calculation:

1x10^8 = 100 000 000 IU/mL

HCV NOT DETECTED

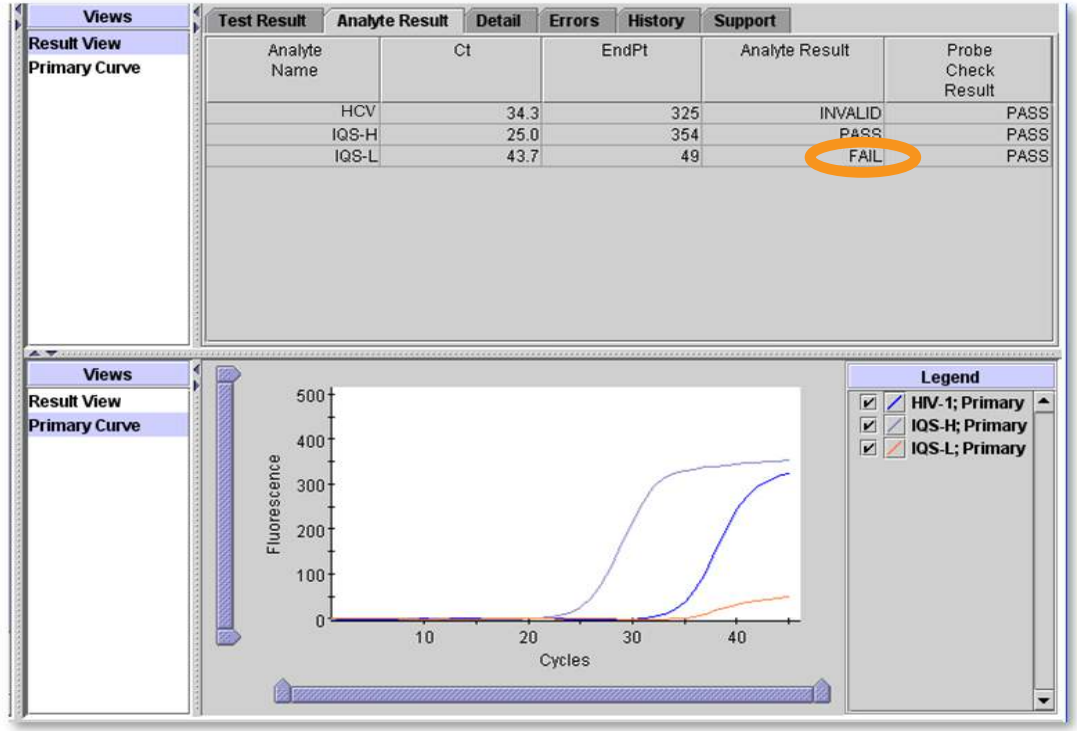
Test Result	HCV NOT DETECTED



- The target HCV is not detected
- 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

INVALID Result

Test Result	INVALID



- Presence or absence of the HCV RNA cannot be determined
- IQS-H and/or IQS-L: FAIL
  - Internal Quantitative Control Cycle thresholds are not within the valid range
  - The endpoint is below the minimum setting
- Probe Check: PASS

Patient Trend Report

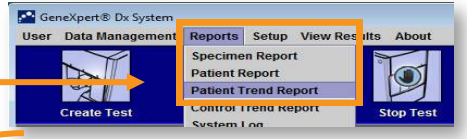
- **You can monitor a specific patient over a period of time by creating a trend report**

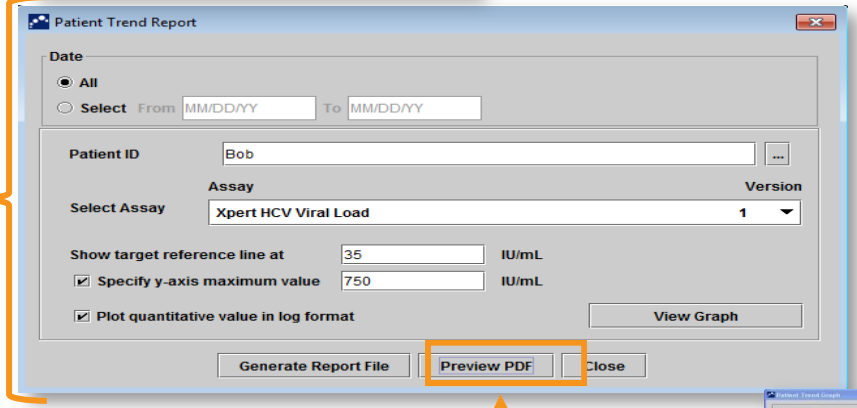
1 Select Reports and Patient Trend Report

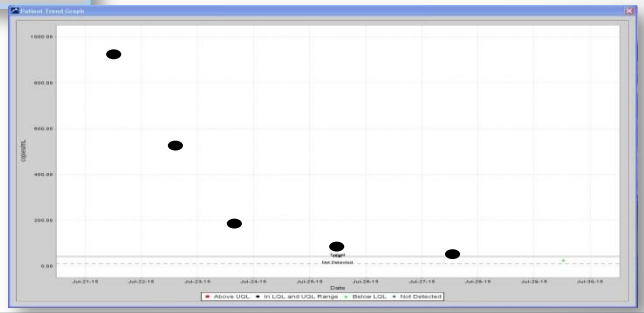
2 Choose
- Time range
- Patient ID
- Assay
- Target preferences

4 Preview the Report

5 View your Report







Available only in Software version GxDx 4.6a and higher

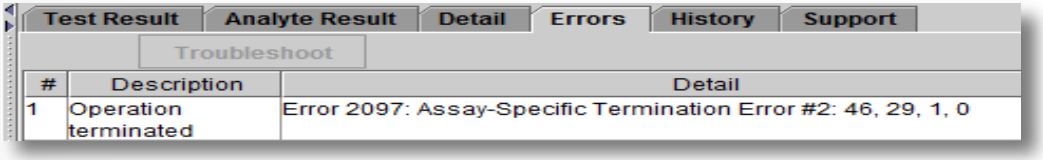
Troubleshooting

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
  - Storage and transport conditions are specimen specific
  - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
  - Modification to the testing procedures may alter the performance of the test
  - Careful compliance with the package insert is necessary to avoid erroneous results

Errors 2096/2097

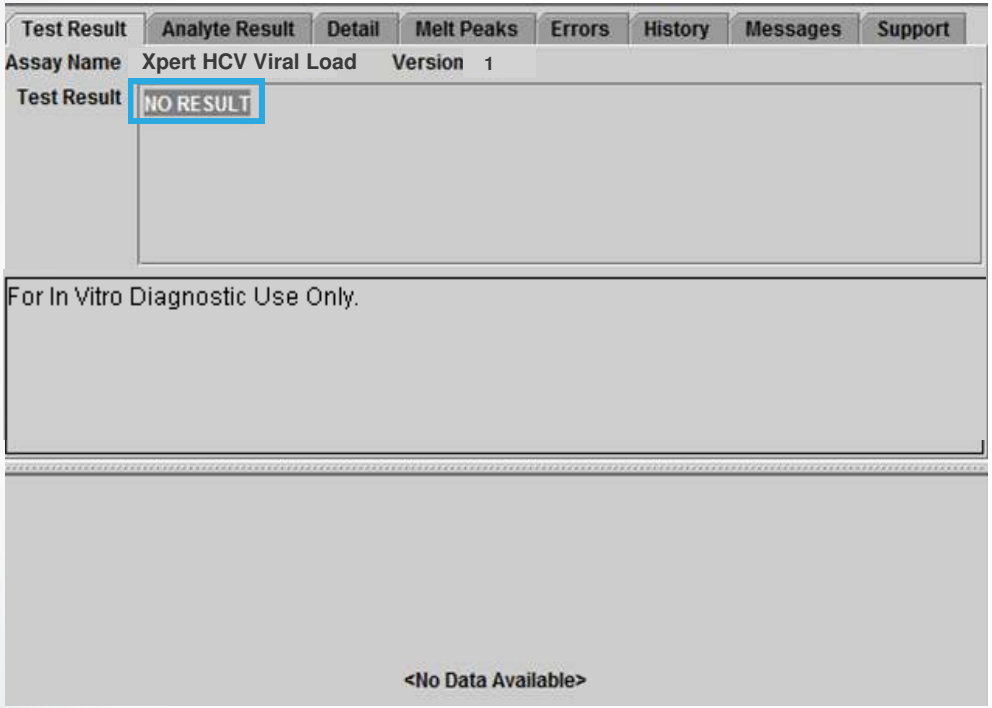
- **The Sample Volume Adequacy (SVA) verifies that the correct volume of sample was added to the cartridge**



Error Code	Reason	Solution
2096	No sample added	Ensure the Sample is added to cartridge
2097	Not enough sample added	Ensure the minimum sample volume is added to the cartridge

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

Xpert® HCV Viral Load Interference

- **Potentially Interfering Substances**
  - A total of 5 endogenous substances were evaluated
  - Elevated levels of those endogenous substances were shown not to impact the assay specificity or interfere with the HCV VL detection

Substance	Tested Concentration
Albumin	9 g/dL
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Human DNA	0.4 mg/dL
Triglycerides	3000 mg/dL

  - The drug components below were shown not to interfere with the quantitation or the specificity of the Xpert HCV VL assay

Pool	Drugs
Control	N/A
1	Zidovudine, Saquinavir, Ritonavir, Interferon alfa-2b, Clarithromycin
2	Abacavir sulfate, Fosamperavir Calcium, Peginterferon 2b, Ribavirin
3	Tenofovir disoproxil fumarate, Lamivudine (3TC), Indinavir sulfate, Ganciclovir, Valganciclovir HCl, Acyclovir
4	Stavudine (d4T), Efavirenz, Lopinavir, Enfuvirtide (T-20), Ciprofloxacin
5	Nevirapine, Nelfinavir mesylate, Azithromycin, Valacyclovir HCl

Re-test Procedure

1

Discard used cartridge

Follow your institution's safety guidelines for disposal of cartridges

2

Obtain the residual sample, mix according to 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

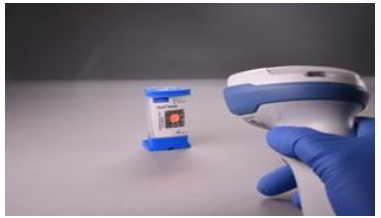
3

Obtain a new cartridge

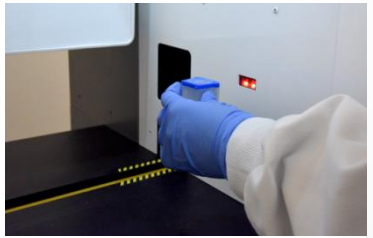
Label appropriately as retest on the new cartridge

Process the sample per the package insert

4



Run the test on the System



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 107 884 (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
Japan	+ 0120 95 4886	support@japan.cepheid.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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