GeneXpert
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Xpert® HCV Viral Load

REF **GXHCV-VL-CE-10**

GXHCV-VL-IN-10

Cepheid *In Vitro* Diagnostic Medical Device CE 2797 IVD 301-3019, Rev. K December 2022

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Xpert® HCV Viral Load

For *In Vitro* Diagnostic Use Only.

1 Proprietary Name

Xpert® HCV Viral Load

2 Common or Usual Name

HCV VL

3 Intended Use

The 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 HCV VL assay quantifies HCV genotypes 1–6 over the range of 10 to 100,000,000 IU/mL. The 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 nonsustained virological responses to HCV therapy.

Results from the 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 Xpert HCV VL assay is intended to be used by laboratory professionals or specifically-trained healthcare workers.

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

4 Summary and Explanation

HCV is a member of the Flaviviridae family and has been recognized as the major causative agent of chronic liver disease, including chronic active hepatitis, cirrhosis and hepatocellular carcinoma.[1] The HCV genome is a positive-sense RNA molecule of approximately 9500 nucleotides.[1] HCV is usually transmitted through percutaneous exposure to infected blood, primarily by intravenous drug use and receipt of unscreened donated blood products. Less frequently, HCV has been shown to be transmitted through occupational, perinatal and sexual exposures.[2]

An estimated 185 million people, or roughly 3% of the world's population, have been infected with HCV, and over 80% live in Low and Middle Income Countries (LMICs).[3] The burden of disease is greatest in developing countries; the highest reported prevalence is in China (3.2%)[4] Pakistan (4.8%)[4], Nigeria (18.3%)[5] and Egypt (22%).[4] About 15 million European adults are infected with HCV and most of these people are unaware of their infection.[6] Each year, 350,000 to 500,000 people die from HCV-related liver disease.[7]

Antiviral medicines can cure HCV, but access to diagnosis and treatment is low.[7] A cure for HCV infection is now possible in most patients with highly effective, safe and tolerable combinations of oral direct-acting antivirals (DAAs) taken for 8–24 weeks.[5] Eradication of HCV is being discussed for the first time.[5]

Quantitation of HCV RNA has proven useful in providing a metric to evaluate the effectiveness of antiviral response to HCV treatment. Guidelines for the management and treatment of HCV recommend quantitative testing for HCV RNA before the start of antiviral therapy, during therapy, and after the conclusion of treatment. The primary objective of treatment is Sustained Virologic Response (SVR), defined as undetectable HCV RNA by a sensitive test 12 or 24 weeks after the end of treatment depending on the anti-HCV therapy.[8]

5 Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using RT-PCR which uses fluorescence to detect the RNA of interest. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the RT-PCR reagents and host the RT-PCR processes. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx Operator Manual* or *GeneXpert Infinity Operator Manual*.

The HCV VL assay includes reagents for the detection of HCV RNA in specimens as well as two internal controls used for quantitation of HCV RNA. The internal controls monitor recovery and the presence of inhibitor(s) in the RT and PCR reactions. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

6 Reagents

6.1 Materials Provided

The HCV VL assay kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

HCV VL Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent (Guanidinium Thiocyanate)	2.0 mL per cartridge
• Rinse Reagent	0.5 mL per cartridge
• Elution Reagent	1.5 mL per cartridge
• Binding Reagent	2.4 mL per cartridge
• Proteinase K Reagent	0.48 mL per cartridge
Disposable 1 mL Transfer Pipettes	**10 per kit**
CD	**1 per kit**
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert software	
• Instructions for Use (Package Insert)	

Note Safety Data Sheets (SDS) are available at 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.

7 Storage and Handling

- Store the HCV VL assay cartridges and reagents at 2–28 °C.
- Do not open the cartridge until you are ready to perform the assay.
- Do not use a cartridge that has leaked.
- Do not use HCV VL assay cartridges and reagents that were previously frozen.
- Do not use reagents or cartridges that have passed the expiration date.

8 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert Instrument, computer with proprietary GeneXpert Dx Software Version 4.7b or higher (GeneXpert Dx systems); or Xpertise 6.4b or higher (Infinity-80/Infinity-48s), barcode scanner, and operator manual.
- Printer: If a printer is needed, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Bleach or sodium hypochlorite

9 Warnings and Precautions

- 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[9] and the Clinical and Laboratory Standards Institute.[10]
- Good laboratory practices and changing gloves between handling specimens are recommended to avoid contamination of specimens or reagents.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute HCV VL assay reagents with other reagents.
- Do not open the HCV VL assay cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake 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.
- Each single-use HCV VL assay cartridge is used to process one test. Do not reuse cartridges.
- The single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes.
- Wear clean lab coats and gloves. Change gloves between processing each sample.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a solution of 1:10 dilution of household chlorine bleach or sodium hypochlorite and then 70% ethanol or 70% denatured ethanol. Wipe work surfaces dry completely before proceeding.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges and unused reagents. Check state, territorial, or local regulations as they may differ from national disposal regulations. The material may exhibit characteristics of hazardous waste requiring specific disposal requirements. Institutions should check their hazardous waste disposal requirements.
- 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 disposal. If country 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.

10 Chemical Hazards[11,12]

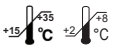
- Signal Word: WARNING
- **UN GHS Hazard Statements:**
  - Harmful if swallowed
  - Causes mild skin irritation
  - Causes eye irritation
- **UN GHS Precautionary Statements:**
  - **Prevention:**
    - Wash thoroughly after handling.
  - **Response:**
    - Call a POISON CENTER of doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - 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.

11 Specimen Collection, Storage, and Transport

Whole blood should be collected in K2-EDTA tubes, EDTA-PPT or serum collection tubes and centrifuged to separate the plasma/serum and red blood cells per the manufacturer's instructions.

- A minimum of 1 mL plasma or serum is required for the HCV VL assay. If using the transfer pipette included in the kit, a minimum of 1.2 mL plasma or serum is required. Alternatively, if using a precision pipette, a minimum of 1 mL plasma or serum is required.

- Whole blood may be held at 15–30 °C for up to 24 hours or at 2–8 °C for up to 3 days prior to plasma/serum preparation. Centrifugation should be performed according to the manufacturer instructions.



- After centrifugation and separation, plasma and serum may be held at 15–35 °C for up to 24 hours or at 2–8 °C for up to 3 days prior to testing.

- Plasma and serum specimens are stable frozen (-70 to -18 °C) for 6 weeks.
- Plasma and serum specimens are stable for up to three freeze/thaw cycles.
- Plasma and serum specimens must be thawed and equilibrated to room temperature prior to transfer to the cartridge.

- Ship whole blood, plasma or serum specimens at 2–8 °C.
- Transportation of whole blood, plasma or serum specimens must comply with country, federal, state and local regulations for the transportation of etiologic agents.

12 Procedure

12.1 Preparing the Specimen

1. Following centrifugation of whole blood samples, 1 mL of plasma can be pipetted directly into the cartridge. Sufficient volume is critical to obtaining valid test results (see instructions in Section 12.2, Preparing the Cartridge Option 1 below).

2. If using frozen specimens, place the specimens at room temperature (20–35 °C) until completely thawed and equilibrated to room temperature before use.

3. Plasma and serum samples stored at 2–8 °C should be removed from the refrigerator and equilibrated to room temperature before use.

4. Plasma samples stored at 2–8 °C or frozen and thawed should be vortexed for 15 seconds before use, if the specimen is cloudy, clarify by a quick centrifugation.

12.2 Preparing the Cartridge

1. Wear protective disposable gloves.
2. Inspect the test cartridge for damage. If damaged, do not use it.
3. Open the lid of the test cartridge.

- **Option 1**: If using the transfer pipette included in the kit (Figure 1), fill to just below the bulb but above the line to transfer at least 1 mL plasma or serum from the collection tube into the sample chamber of the test cartridge (Figure 2). Do **NOT** pour the specimen into the chamber!
- **Option 2**: If using an automatic pipette, transfer at least 1 mL of plasma or serum into the sample chamber of the test cartridge (Figure 2). Do **NOT** pour the specimen into the chamber!

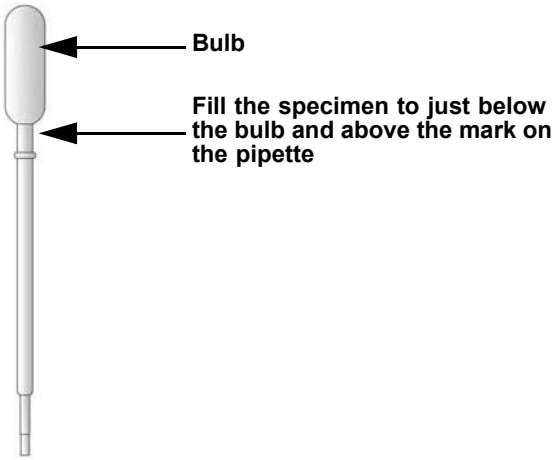


Figure 1. HCV VL Assay Transfer Pipette

4. Close the cartridge lid.
5. Load the cartridge into the GeneXpert Dx instrument or Infinity system.

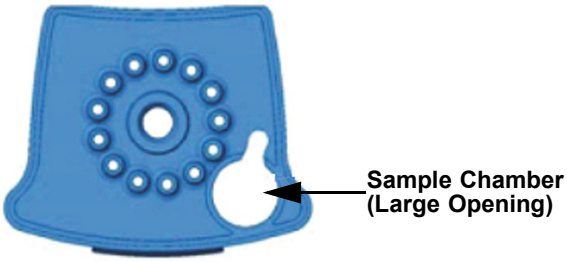


Figure 2. HCV VL Assay Cartridge (Top View)

12.3 Starting the Test

Important **Before you start the test, make sure the HCV VL Assay Definition File (ADF) is imported into the software.**

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

1. Turn on the GeneXpert instrument:
   - If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically. If it doesn't, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. The GeneXpert software will launch automatically. If it doesn't, double-click the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** and **Order Test** (Infinity).
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all reports. The Scan Cartridge dialog box appears.
6. Scan the barcode on the HCV VL assay cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). Enter your password, if requested.
8. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

   or

   For the GeneXpert Dx Instrument:

   A. Open the instrument module door with the blinking green light and load the cartridge.

   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.

   C. Wait until the system releases the door lock before opening the module door and removing the cartridge.

   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution’s standard practices.

13 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the instrument used.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

14 Quality Control

CONTROL

Each test includes a Sample Volume Adequacy (SVA), Internal Quantitative Standard High and Low (IQS-H and IQS-L, also acts a specimen processing control [SPC]) and Probe Check Control (PCC).

- **Sample Volume Adequacy (SVA) –** Ensures the sample was correctly added to the cartridge. The SVA verifies that the correct volume of sample has been added in the sample chamber. The SVA passes if it meets the validated acceptance criteria. If the SVA does not pass, an **ERROR 2096** will be displayed if there is no sample or an **ERROR 2097** will be displayed if there is not enough sample. The system will prevent the user from resuming the test.
- **Internal Quantitative Standard High and Low (IQS-H and IQS-L) –** IQS-H and IQS-L are two Armored RNA® constructs in the form of a dry bead that goes through the whole assay process. The IQS-H and IQS-L are standards calibrated against the WHO 4th International standard for HCV. They are used for quantification by using lot specific parameters for the calculation of HCV RNA concentration in the sample. Additionally IQS-H and IQS-L detect specimen-associated inhibition of the RT-PCR reaction. The IQS-H and IQS-L pass if they meet the validated acceptance criteria.
- **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. The PCC passes if it meets the validated acceptance criteria.
- **External Controls –** Following good laboratory practices, external controls, not available in the kit, should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable.

15 Interpretation of Results

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window (Figure 3 and Figure 5). Possible results are shown in Table 1.

Table 1. HCV VL Assay Results and Interpretation

Result	Interpretation
HCV DETECTED **XX IU/mL (log X.XX)** See Figure 3.	The HCV RNA is detected at XX IU/mL. • The HCV RNA has a titer within the linear range setting of the assay and the endpoint above the minimum. • IQS-H and IQS-L: PASS. • Probe Check: PASS; all probe check results pass.
HCV DETECTED **> 1.00E08 IU/mL** See Figure 4.	The HCV RNA is detected above the quantitative range of the assay. • IQS-H and IQS-L: PASS. • Probe Check: PASS; all probe check results pass.
HCV DETECTED **< 10 IU/mL** See Figure 5.	The HCV RNA is detected below the quantitative range of the assay. • IQS-H and IQS-L: PASS. • Probe Check: PASS; all probe check results pass.
HCV NOT DETECTED See Figure 6.	The HCV RNA is not detected. • HCV RNA is not detected. • IQS-H and IQS-L: PASS. • Probe Check: PASS; all probe check results pass.
INVALID See Figure 7.	Presence or absence of the HCV RNA cannot be determined. Repeat test according to the instructions in Section 16.2, Retest Procedure. • IQS-H and/or IQS-L: FAIL; Cycle thresholds (Cts) are not within valid range and the endpoint is below the minimum setting. • Probe Check: PASS; all probe check results pass.

Table 1. HCV VL Assay Results and Interpretation (Continued)

Result	Interpretation
ERROR See Figure 8.	Presence or absence of HCV RNA cannot be determined. Repeat test according to the instructions in Section 16.2, Retest Procedure. • Probe Check: FAIL*; all or one of the probe check results fail. * If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	Presence or absence of HCV RNA cannot be determined. Repeat test according to the instructions in Section 16.2, Retest Procedure. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Note Assay screenshots are for example only. Assay name and version number may vary from the screenshots shown in this package insert.

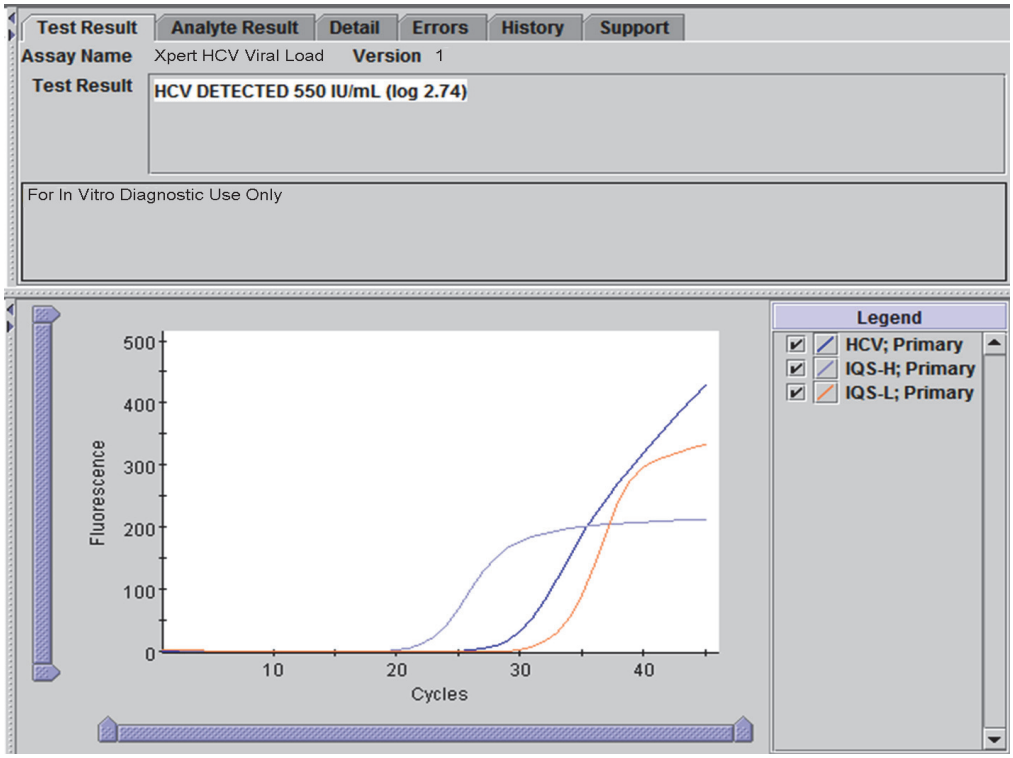


Figure 3. HCV Detected and Quantified

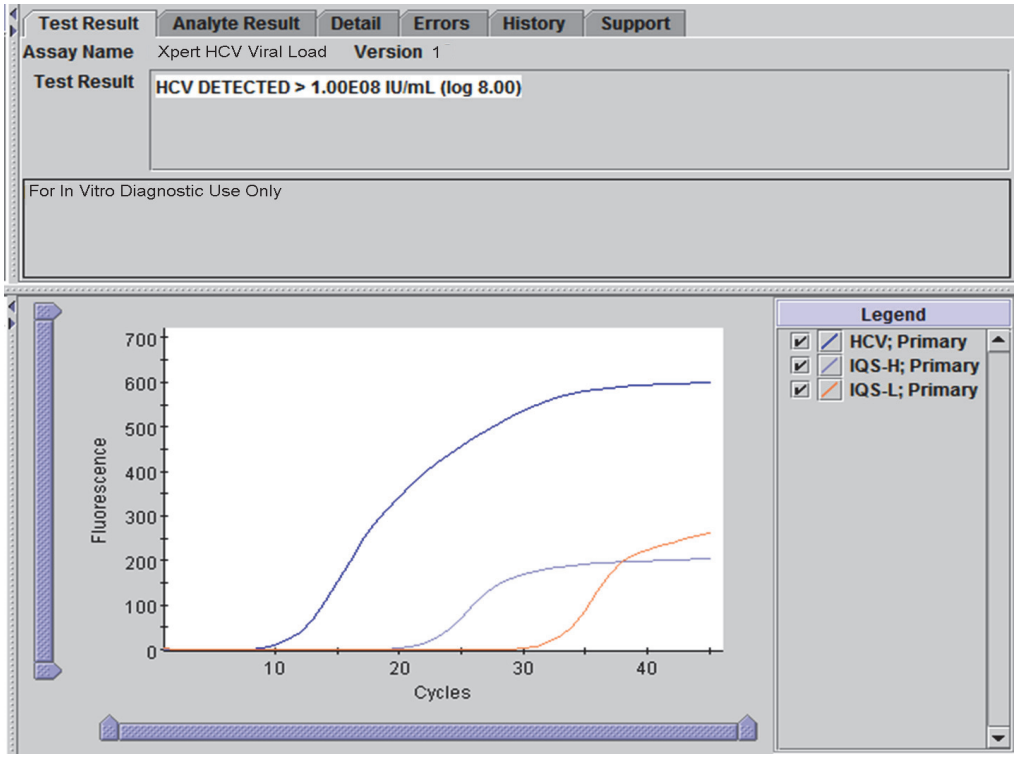


Figure 4. HCV Detected

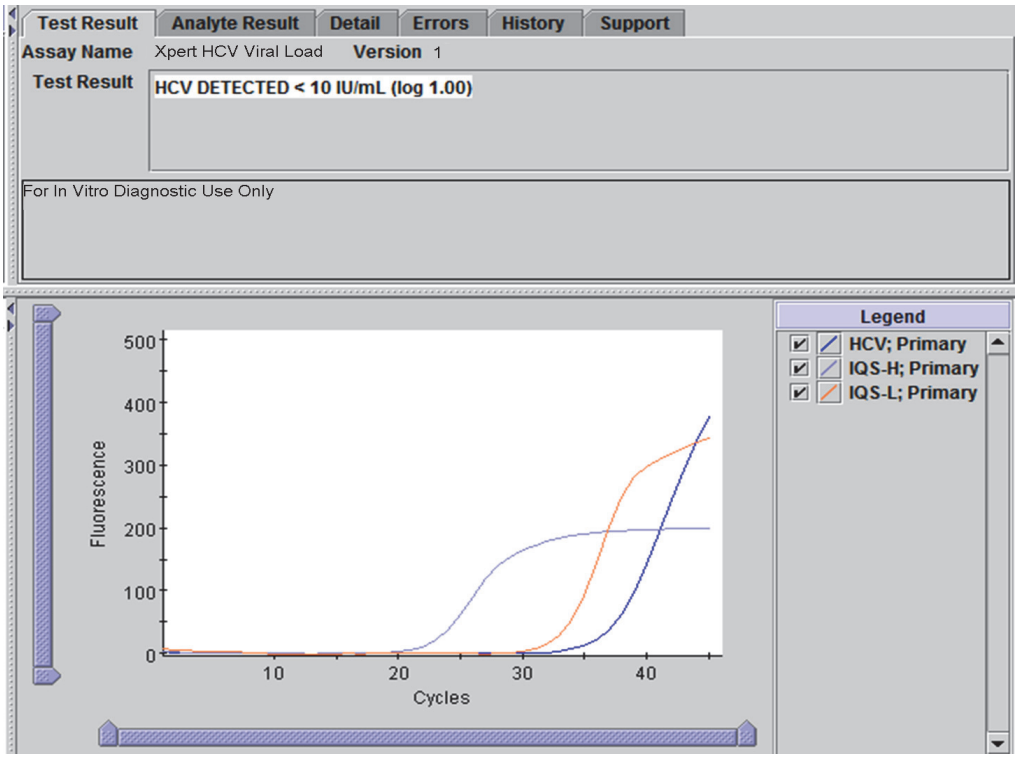


Figure 5. HCV Detected

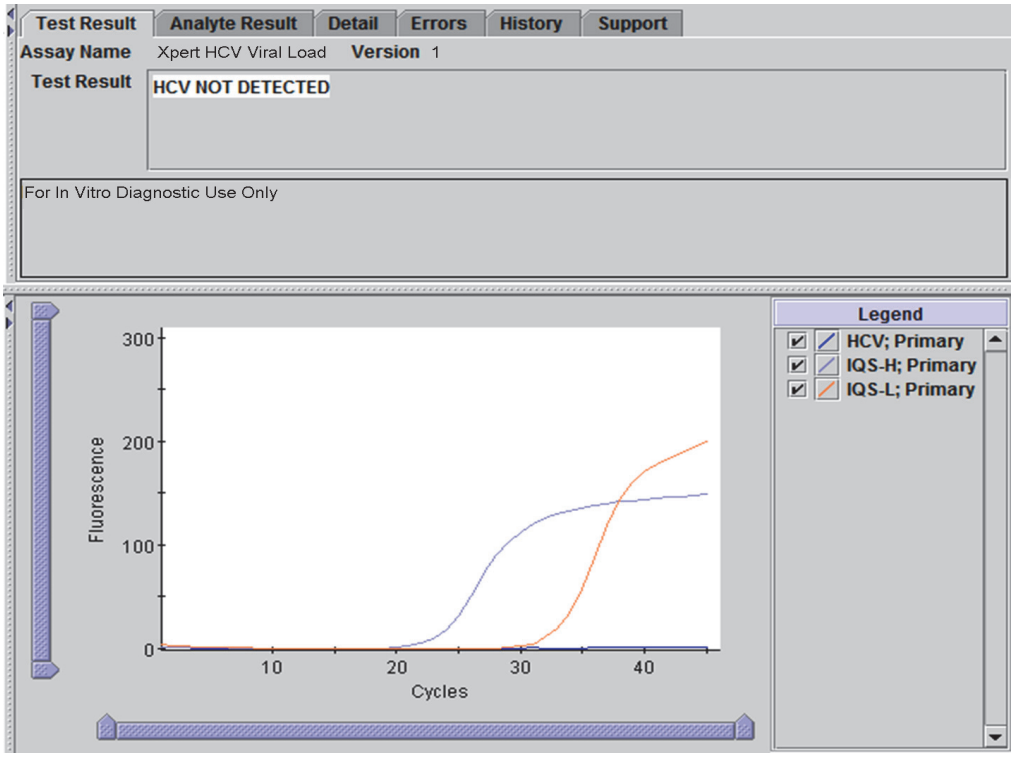


Figure 6. HCV Not Detected

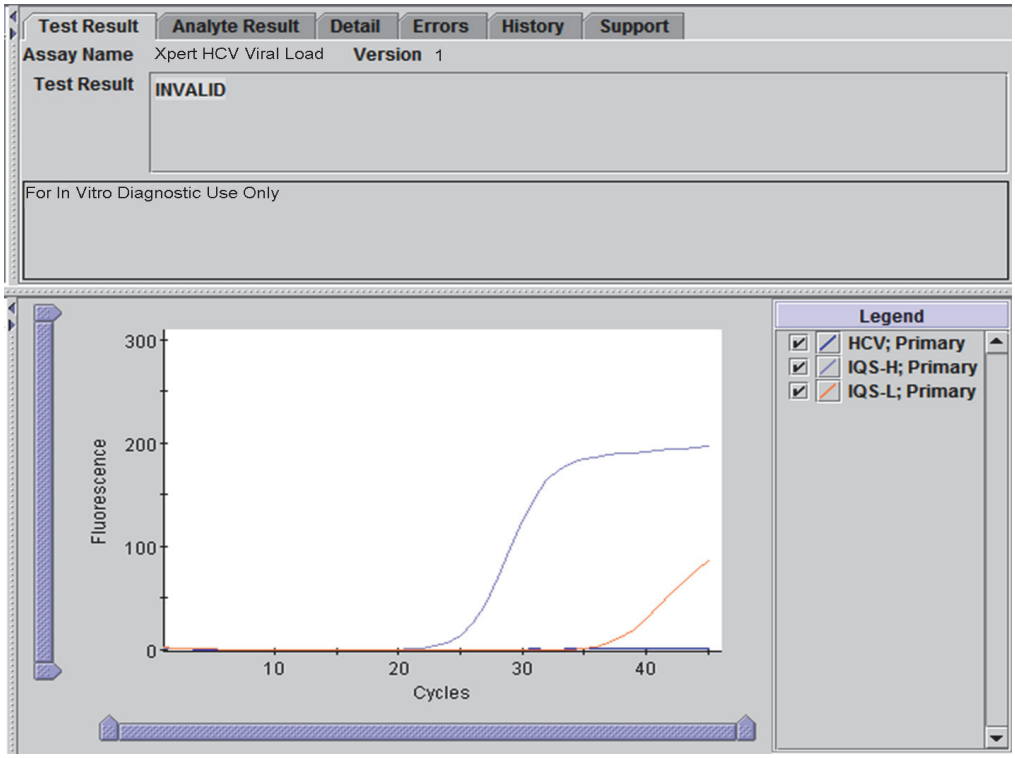


Figure 7. Invalid

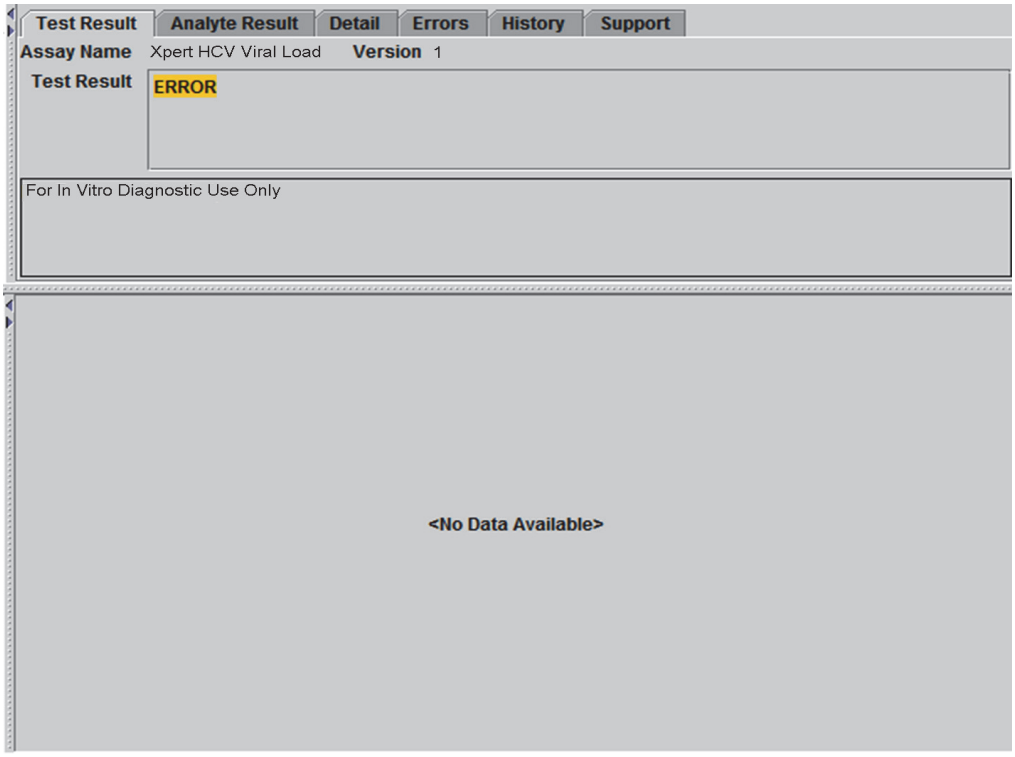


Figure 8. Error

16 Retests

16.1 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to the instructions in Section 16.2, Retest Procedure.

- An **INVALID** result indicates one or more of the following:
  - The IQS-H and/or IQS-L Cts are not within valid range.
  - The sample was not properly processed or PCR was inhibited.
- An **ERROR** result indicates that the assay was aborted. Possible causes include: insufficient volume of sample was added, the reaction tube was filled improperly, a reagent probe integrity problem was detected, or the maximum pressure limit was exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or a power failure occurred.

16.2 Retest Procedure

For retest of a **NO RESULT**, **INVALID**, or **ERROR** result, use a new cartridge (do not re-use the cartridge) and new reagents.

1. Remove a new cartridge from the kit.
2. See Section 12, Procedure, including Section 12.1, Preparing the Specimen, Section 12.2, Preparing the Cartridge, and Section 12.3, Starting the Test.

17 Limitations

Good laboratory practices and changing gloves between handling specimens are recommended to avoid contamination of reagents.

Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown HCV variants resulting in a false negative result.

18 Performance Characteristics

18.1 Limit of Detection

The limit of detection (LOD) of the HCV VL assay was determined by testing eight different dilutions prepared from a HCV genotype 1 reference standard in HCV negative EDTA plasma and serum. The HCV genotype 1 material used in the LOD study was the WHO 4th International standard, NIBSC code 06/102. The limit of detection was determined for three reagent lots and a total of 72 or 73 replicates per concentration level were tested. One additional low concentration level was included for both sample types after the first day of testing. The number of tested replicates for this level was thus smaller (49 in plasma and 53 in serum). The evaluation was performed according to CLSI guideline E17-A2. The HCV RNA concentration that can be detected with a positivity rate of greater than 95% was determined by Probit regression analysis and the results for the individual lots and specimens are shown in Table 2. The maximum observed LOD with Probit analysis for HCV genotype 1 in EDTA plasma is 4.0 IU/mL (95% CI 2.8 – 5.2). The maximum observed LOD with Probit analysis for HCV genotype 1 in serum is 6.1 IU/mL (95% CI 4.2 – 7.9).

Table 2. HCV VL LOD Estimates with Probit Regression and 95% Upper and Lower Confidence Intervals for HCV Genotype 1 Specimens in Plasma and Serum per Kit Lot

Specimen	Lot	LOD 95% (IU/mL)	95% CI (IU/mL)
WHO (Plasma)	1	3.3	2.4 - 4.2
	2	4.0	2.7 - 5.2
	3	4.0	2.8 - 5.2
WHO (Serum)	1	6.1	4.2 - 7.9
	2	2.6	1.9 - 3.3
	3	2.3	1.8 - 2.9

Hit rate analysis shows a positivity of > 95% at 6 IU/mL for the HCV genotype 1 material tested as shown in Table 3.

Table 3. HCV VL LOD for HCV Genotype 1 in EDTA Plasma and Serum

Specimen	Concentration (IU/mL)	No. Replicates	No. Positives	Positivity Rate (%)
WHO (Plasma)	0.5[a]	49	24	49
	1	72	47	65
	2	72	61	85
	3	72	69	96
	4	72	67	93
	6	72	71	99
	8	73	73	100
	10	72	72	100
WHO (Serum)	0.5[a]	53	21	40
	1	73	47	64
	2	73	64	88
	3	72	69	96
	4	73	71	97
	6	72	71	99
	8	72	70	97
	10	72	72	100

a. 0.5 IU/mL was added day 2 due to the high positivity rate observed at 1 IU/mL after day 1

In addition, dilutions of clinical specimens representing HCV genotype 1a, 2b, 3a, 4a, 5a and 6a in negative human EDTA plasma were analyzed with one reagent lot and 24 replicates per concentration level. The assignment of the nominal concentration of clinical specimens was determined by Abbott RealTime HCV™ assay. Hit rate analysis shows a positivity of >95% for all genotypes at 10 IU/mL as shown in Table 4.

Table 4. HCV VL LOD Hit Rate Analysis for HCV Genotype 1 – 6 Specimens in EDTA Plasma

Genotype	Lowest Concentration Level > 95% Hit Rate (IU/mL)	Hit Rate (%)
1a	10	100
2b	4	100
3a	6	100
4a	4	100
5a	2	96
6a	4	96

18.2 Limit of Quantitation

The total analytical error (TAE) was calculated using estimates determined through analysis of data from LOD study (WHO standard) and the Precision/Reproducibility study according to CLSI guideline E17-A2. The TAE for the dilutions that had an observed concentration at or near the assay limit of detection 10 IU/mL (1.0 $\log_{10}$) are presented in Table 5. TAE was estimated by two different methods.

Table 5. HCV VL TAE Analysis for Determination of LOQ

Specimen (Study)	DL Lot	N	Concentration (Log10 IU/mL)		Bias	Total SD	TAE[a] Absolute Bias + 2xSD	TAE[b] 2xSQRT (2)xSD
			Expected	Observed				
Acrometrix (Precision)	DL1	72	1.40	1.31	0.09	0.15	0.38	0.41
	DL2	72	1.40	1.29	0.11	0.14	0.40	0.41
	DL3	72	1.40	1.24	0.16	0.12	0.41	0.35
Acrometrix (Precision)	DL1	72	1.00	0.92	0.08	0.22	0.52	0.62
	DL2	72	1.00	0.82	0.18	0.18	0.54	0.51
	DL3	72	1.00	0.75	0.25	0.19	0.63	0.54
WHO, Plasma (LOD)	DL1	24	1.00	0.91	0.09	0.21	0.51	0.59
	DL2	24	1.00	0.82	0.18	0.30	0.78	0.86
	DL3	24	1.00	0.86	0.14	0.17	0.48	0.48
WHO, Serum (LOD)	DL1	24	1.00	0.96	0.04	0.13	0.30	0.37
	DL2	24	1.00	0.88	0.12	0.23	0.58	0.66
	DL3	24	1.00	0.80	0.20	0.18	0.57	0.52

a. TAE calculated according to the Westgard model in CLSI EP17-A2 (Section 6.2)
b. TAE based upon the difference between two measurements approach

The results of the TAE analysis demonstrate that the HCV VL assay can determine 10 IU/mL (1.0 $\log_{10}$) with an acceptable trueness and precision.

18.3 Precision/Reproducibility

The precision/reproducibility of the HCV VL assay was determined by analysis of parallel dilutions of HCV reference materials in HCV negative EDTA plasma. The nominal concentration of the reference material used was calibrated to the WHO 4th HCV International Standard (06/102). The study was a two site, blinded, comparative study using a seven-member panel of HCV reference material in HCV negative EDTA plasma with RNA concentrations that span the HCV VL assay quantitation range. Two operators at each of the two study sites tested one panel of twenty-one samples once per day over six testing days per lot. One site used an Infinity-80 instrument and the other site used GeneXpert Dx instruments. Three lots of HCV VL assay reagents were used for the study. Precision/reproducibility was evaluated in accordance with "Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline" CLSI document EP5-A2. The precision results for each regent lot are shown in Table 6.

Table 6. HCV VL Precision per Lot

Expected HCV RNA Concentration log_{10} IU/mL	Total Precision per Lot					
	Lot 1		Lot 2		Lot 3	
	SD	CV[a]	SD	CV[a]	SD	CV[a]
1.0	0.23	55.8%	0.18	44.2%	0.20	48.1%
1.4	0.15	35.1%	0.15	35.8%	0.13	29.6%
2.7	0.09	20.7%	0.09	20.6%	0.09	20.2%
4.2	0.07	16.4%	0.08	18.9%	0.07	15.3%
5.4	0.12	28.3%	0.09	19.9%	0.07	16.2%
6.9	0.13	31.8%	0.09	20.9%	0.07	17.0%
8.2	0.10	22.7%	0.10	23.7%	0.08	17.8%

a. "CV" is lognormal CV, as obtained using the formula:

$$CV(\text{of the lognormal dist}) = \sqrt{10^{\ln(10)*\sigma^2} - 1}$$

The reproducibility and precision of the HCV VL assay was evaluated by using nested ANOVA with terms for Site/Instrument, Lot, Day, Operator/Run and Within-Run. The standard deviation and the percentage of variability due to each component of the log_{10} HCV transformed concentrations were calculated, see Table 7.

Table 7. Standard Deviation and Contributable Percentage of Variability for Each Term and Total Precision

HCV RNA Concentration log_{10} IU/mL			Contribution to Total Variance SD (CV%)										Total Precision			
			Site/Inst		Lot		Day		Operator/ Run		Within-Run		Total			
Expected	Actual	N	SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	Lower CI	Upper CI	CV[b]
1.0	0.83	216	0.03	1.8%	0.08	13.2%	0.04	3.5%	0.00	0.0%	0.19	81.6%	0.21	0.18	0.25	51.7%
1.4	1.28	216	0.00	0.0%	0.04	7.1%	0.00	0.0%	0.00	0.0%	0.14	92.9%	0.14	0.13	0.16	34.1%
2.7	2.66	216	0.00	0.0%	0.04	17.2%	0.00	0.0%	0.02	3.2%	0.08	79.5%	0.09	0.08	0.11	22.1%
4.2	4.18	215	0.00	0.0%	0.05	30.9%	0.01	2.6%	0.00	0.0%	0.07	66.5%	0.09	0.07	0.12	20.6%
5.4	5.44	216	0.00	0.0%	0.06	26.5%	0.00	0.0%	0.01	1.3%	0.09	72.2%	0.11	0.09	0.14	25.8%
6.9	6.86	216	0.00	0.0%	0.07	34.0%	0.02	3.4%	0.00	0.0%	0.10	62.5%	0.13	0.10	0.17	29.8%
8.2	8.11	216	0.00	0.0%	0.09	47.9%	0.00	0.0%	0.02	2.6%	0.09	49.5%	0.13	0.10	0.19	30.5%

a. (%) is contribution of variance component to overall lognormal CV
b. "CV" is lognormal CV, as obtained using the formula:

$$CV(\text{of the lognormal dist}) = \sqrt{10^{\ln(10)*\sigma^2} - 1}$$

18.4 Linear Range and Inclusivity

The linear range of the HCV VL assay was determined by analysis of a twelve member panel covering a range from ~5 (0.75 $\log_{10}$) to ~1 x 10^8 (8 $\log_{10}$) IU/mL. Panels were prepared by parallel dilutions of HCV reference material (Armored RNA® genotype 1 and clinical specimen genotype 1) in HCV negative EDTA plasma and serum. The nominal concentration of the reference material used was calibrated to the WHO 4th HCV International Standard (06/102). Each panel member was tested in replicates of four on each of three testing days using two kit lots. Totally, 24 replicates per panel member and sample type were tested. The linearity analysis was performed according to CLSI guideline EP06-A. The combined results for both lots are shown in Figure 9 and Figure 10. The HCV VL assay is linear within a range 0.8–8.0 $\log_{10}$ IU/mL with a R^2 value of >0.997.

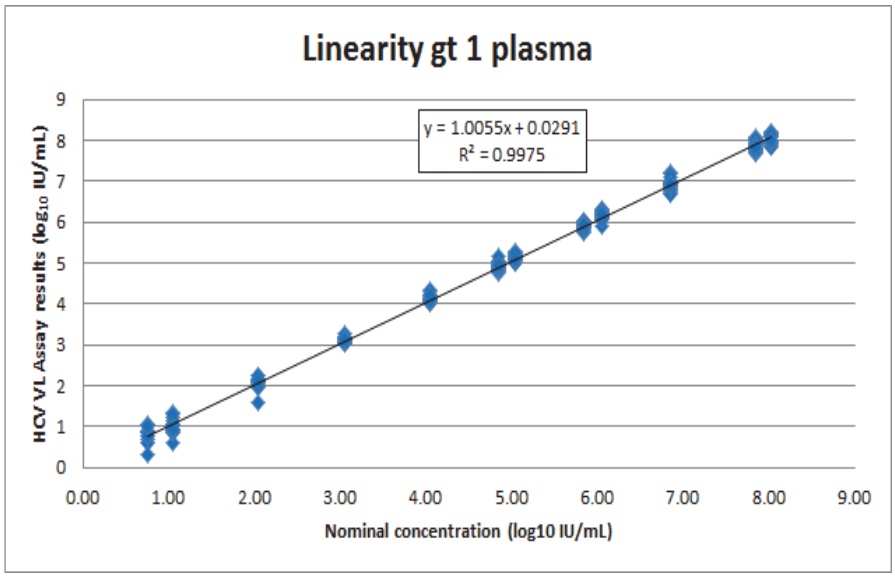


Figure 9. Linearity Genotype 1 in EDTA Plasma for the HCV VL Assay

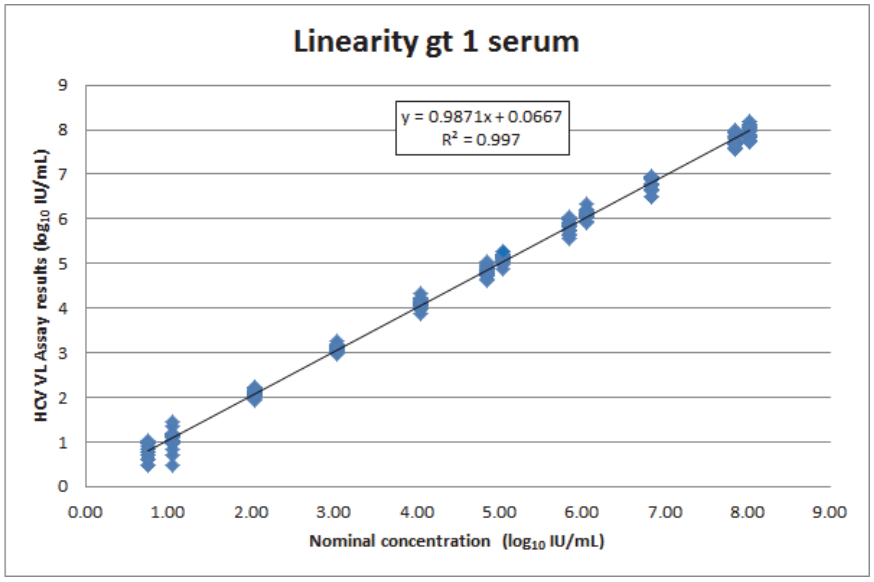


Figure 10. Linearity Genotype 1 in Serum for the HCV VL Assay

To confirm the linear range and evaluate the inclusivity of the HCV VL assay, panels consisting of clinical specimens representing HCV genotype 2 – 6 and Armored RNA® when available (genotypes 2 and 3 only) were prepared in negative human EDTA plasma. 7 – 13 panel members per genotype covering as wide a range as possible, varying from ~ 0.9 – 6 $\log_{10}$ IU/mL for genotype 5 to ~ 0.9 – 8.3 $\log_{10}$ for genotype 3, were prepared and analyzed in replicates of four on each of three testing days using two kit lots. For each genotype, 24 replicates per panel member were tested. The nominal concentrations of the reference materials used were calibrated to the WHO 4th HCV International Standard (06/102). All genotypes responded linearly with R^2 values ranging from 0.994 – 0.998.

18.5 Analytical Specificity (Exclusivity)

The analytical specificity of the HCV VL assay was evaluated by adding potentially cross-reacting organisms at 1 x 10^5 CFU/mL, copies/mL or $TCID_{50}$/mL input concentration into HCV negative EDTA plasma and in plasma that contained ~25 IU/mL HCV reference material (clinical specimen genotype 1). Tested organisms are listed in Table 8.

Table 8. Analytical Specificity Organisms

Human Immunodeficiency virus 1
Human Immunodeficiency virus 2
Human T-cell lymphotropic virus I
Human T-cell lymphotropic virus II
Candida albicans
Cytomegalovirus
Epstein-Barr virus
Hepatitis A virus
Hepatitis B virus
Herpes simplex virus 1
Herpes simplex virus 2
Human herpes virus 6
Human herpes virus 8
Varicella Zoster virus
BK Human polyoma virus
Banzi virus
Ilheus virus
West Nile virus
Zika virus
Human papilloma virus 16
Human papilloma virus 18
Staphylococcus epidermidis
Staphylococcus aureus

None of the tested organisms showed cross reactivity and all positive replicates resulted in concentrations of HCV RNA within ± 0.5 log from a HCV positive control when tested using the HCV VL assay. In addition to the species listed in Table 8, Dengue virus and vaccinia virus were analyzed *in silico* since material representing the viruses could not be obtained for testing. No practical significant sequence similarity was found between the analyzed viruses and the primers and probes of the Xpert HCV VL assay.

18.6 Potentially Interfering Substances

The susceptibility of the HCV VL assay to interference by elevated levels of endogenous substances, by drugs prescribed to HCV infected patients and by autoimmune disease markers was evaluated. HCV negative EDTA plasma and plasma that contained ~25 IU/mL HCV reference material (clinical specimen genotype 1) were tested.

Elevated levels of the endogenous substances listed in Table 9 were shown not to interfere with the quantification of the HCV VL assay or impact the assay specificity.

Table 9. Endogenous Substances and Concentration Tested

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 as presented in Table 10 were shown not to interfere with the quantification of the HCV VL assay or impact the assay specificity when tested at three times peak level concentration in five drug pools.

Table 10. Drug Pools Tested

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

Testing of specimens from ten individuals per autoimmune disease marker shows no interference with the autoimmune disease markers systemic lupus erythematosus (SLE), anti-nuclear antibody (ANA), or rheumatoid factor (RF) using the HCV VL assay.

18.7 Seroconversion Sensitivity

The sensitivity of the HCV VL Assay was evaluated by testing sequential plasma specimens from ten seroconversion panels with a total of 59 panel members. Each seroconversion panel consisted of undiluted plasma specimens collected from a single donor during development of HCV infection and subsequent immune response. The HCV VL assay detected HCV RNA in 51 out of 57 tested specimens with valid test result as compared to 21 of the 59 tested that were detected by at least one of the HCV antibody tests (Abbott ARCHITECT HCV Ab, Abbott PRISM HCV Ab, Ortho® Ver. 3.0 ELISA HCV Ab, Ortho HCV 3.0 ELISA Test System with Enhanced SAVe, Ortho Vitros Eci, Siemens ADIVA Centaur). HCV RNA was detected by the HCV VL Assay prior to antibody tests in nine seroconversion panels and at the same time point for one seroconversion panel. The result is presented in Table 11.

Table 11. Seroconversion Sensitivity of the HCV VL Assay

Panel No	No. of Specimens in Panel	Days Spanned	No. of Reactive Panel Members		Days to First Reactive Result		Days Between First Reactive Result with Xpert HCV VL and Any Ab Test
			Xpert HCV VL	Antibody (Ab) Test[a]	Xpert HCV VL	Antibody (Ab) Test[a]	
PHV913	4	9	4	2	0[b]	7	7
PHV915	4	14	3[c]	2	5[c]	12	7
PHV920	9	35	9	7	0[b]	13	13
PHV922	6	17	5[c]	5	3[c]	3	0
PHV924	6	88	6	3	0[b]	59	59
PHV925	5	27	5	1	0[b]	27	27
PHV926	5	14	5	1	0[b]	14	14
PHV927	5	17	4	0	4	17[d]	13
PHV928	9	50	7	0	29	50[d]	21
PHV929	6	22	3	0	14	22[d]	8

a. Antibody test based on vendor data: Abbott ARCHITECT HCV Ab, Abbott PRISM HCV Ab, Ortho Ver. 3.0 ELISA HCV Ab, Ortho Enhanced SAVe HCV Ab,Ortho Vitros Eci, Siemens ADIVA Centaur.
b. All bleeds were detected with the Xpert HCV VL Assay.
c. All test results of Xpert HCV VL is presented, first panel member caused an invalid test result.
d. All bleeds were non-reactive for HCV Antibodies (based on vendor information). The last bleed day is used as “Days to First Reactive Result”

18.8 Sample Collection Media Equivalence (EDTA, PPT-EDTA and Serum)

For each sample collection media (EDTA, PPT-EDTA and serum) specimens from 50 matched HCV positive individuals and 25 matched HCV negative specimens were collected and tested using one kit lot of the HCV VL assay.

As shown in Figure 11 and Figure 12 equivalent performance of the HCV VL assay was shown for EDTA plasma versus serum samples and EDTA plasma versus PPT-EDTA plasma samples. All HCV positive specimens collected in serum or PPT-EDTA plasma produced concentrations of HCV RNA within ±0.5 $\log_{10}$ IU/mL of the HCV positive specimen collected in EDTA plasma when tested using the HCV VL assay.

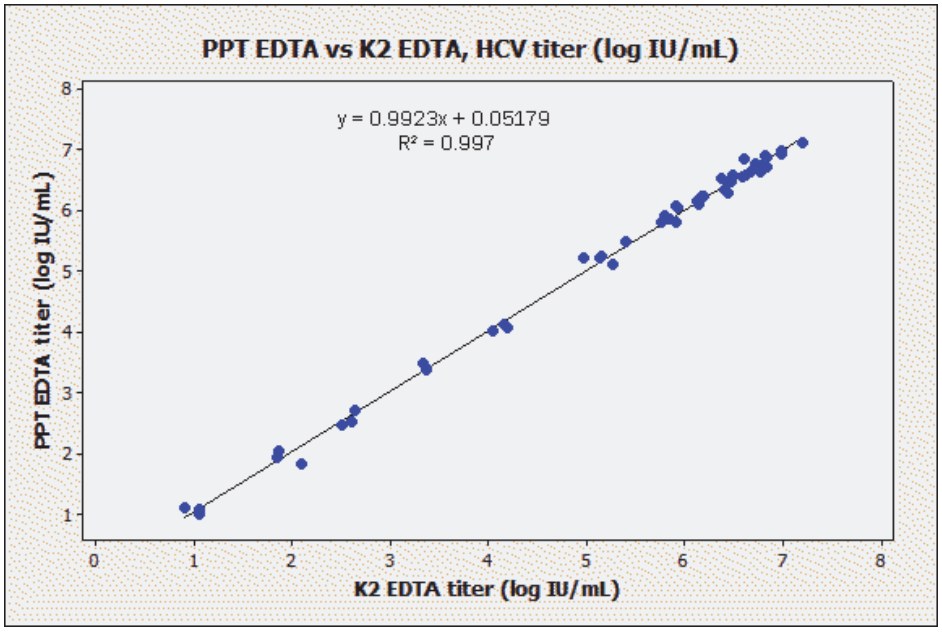


Figure 11. Scatterplot of Log IU/mL PPT-EDTA versus Log IU/mL EDTA

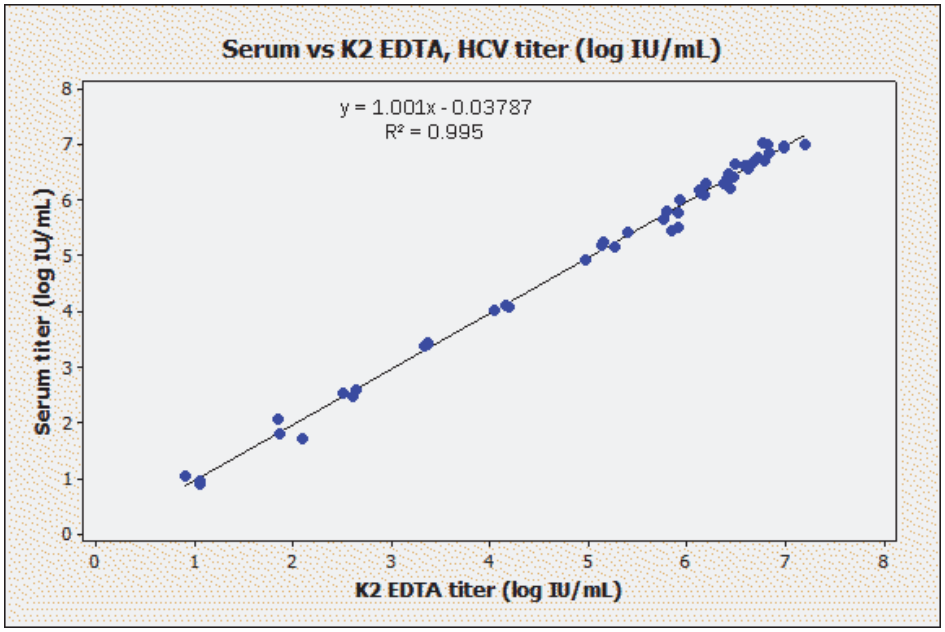


Figure 12. Scatterplot of Log IU/mL Serum versus Log IU/mL EDTA Plasma

19 Performance Characteristics – Clinical Performance

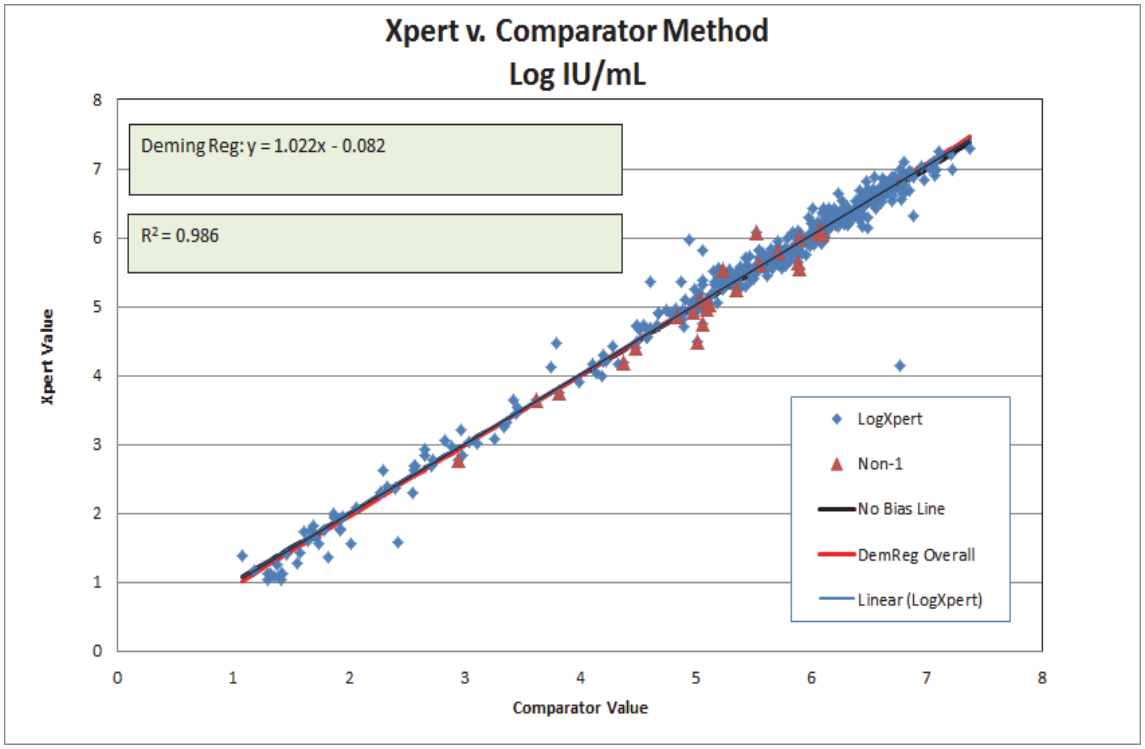
Specificity

The specificity of the HCV VL assay was evaluated using 501 EDTA plasma specimens from HCV negative blood donors. HCV RNA was not detected in any of the 501 specimens tested by the Xpert HCV VL assay demonstrating 100% specificity (95% CI: 99.2-100.0).

Method Correlation

A multi-site study was conducted to evaluate the performance of the HCV VL assay relative to a comparator method using fresh and frozen human plasma or serum specimens collected from HCV infected individuals. Of the 607 eligible specimens, each from unique individuals, 408 (67.2%) were collected from male subjects. The average age was 50.2 ± 13.2 years with an age range of 21 to 86 years.

Of the 607 specimens, 389 were within the quantitation range of both assays including 23 specimens that were HCV non-1 genotypes (2, 2a, 2b, 2c, 3, 3a, 4 & 6) and one mixed genotype (HCV 1 & 6). The Deming regression shows very good correlation between the HCV VL and the comparator method with a slope of 1.022 and intercept of 0.082. The R^2 was 0.986.



*HCV non-1 genotypes are represented as triangles. A single outlier was not included in the analysis.

Figure 13. Xpert v. Comparator Method

20 References

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22 Technical Assistance

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

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

23 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
	Control
	Expiration date
	CE marking – European Conformity
	Temperature limitation
	Biological risks
	Warning
	Authorized representative in Switzerland
	Importer

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