WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: Xpert HCV VL Fingerstick
WHO reference number: PQDx 0453-070-00

Xpert HCV VL Fingerstick with product code GXHCV-FS-CE-10, manufactured by Cepheid AB, CE-Mark, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 5 December 2022.

Summary of WHO prequalification assessment for Xpert HCV VL Fingerstick

	Date	Outcome
Prequalification listing	5 December 2022	listed
Dossier review	Abridged	N/A
Site inspection(s) of the quality management system	25 October 2022	MR
Product performance evaluation	Quarter (Q) 1-Q 3 2021	MR

MR: Meet Requirements
N/A: Not Applicable

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Public report amendment	Summary of the amendments	Date of report amendment
2.0	1. Closing the performance evaluation commitment and including the results in the public report. 2. Addition of the new manufacturing site, Sanmina Penang, Malaysia, for the GeneXpert Dx instruments.	21 October 2025.

Intended use

According to the claim of intended use from Cepheid AB, *"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 FS 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 FS 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.
The Xpert HCV VL FS 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."

Assay description

According to the claim of assay description from Cepheid AB, " *The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using reverse transcription PCR (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, see the appropriate GeneXpert Dx Operator Manual or GeneXpert Infinity Operator Manual. The HCV VL FS assay includes reagents for the detection of HCV RNA in clinical specimens as well as two internal controls used for quantitation of HCV RNA. The internal controls are also used to control for adequate processing of the target and to monitor 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.*
The assay is standardized against the 4th World Health Organization (WHO) International Standard for HCV (NIBSC code: 06/102)."

Test kit contents

Component	**10 tests (product code GXHCV-FS-CE-10)**
HCV VL FS Assay Cartridges with Integrated Reaction Tubes	10 cartridges
CD	1 CD (Assay Definition File (ADF), Instructions to import ADF into GeneXpert software and Instructions for Use (Package Insert)

Items required but not provided

- GeneXpert Dx System or GeneXpert Infinity System (catalogue number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert 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
- Ethanol or denatured ethanol
- Disposable Minivette POCT 100 µL K3E (P/N: MINIVETTE 100E-100, 100 per box)
- Disposable Safety-Lancet Super, 1.5 mm (Sarstedt P/N: 85.1018) or similar.

Storage
The test kit should be stored at 2–28 °C.

Shelf-life upon manufacture
12 months.

Warnings/limitations
Please refer to the attached instructions for use (IFU). Users are not to use specimens subjected to more than two freeze-thaw cycles.

Prioritization for prequalification
Based on the established criteria, Xpert HCV VL Fingerstick was given priority for the WHO prequalification assessment.

Product dossier assessment

In accordance with the WHO procedure for abridged prequalification assessment,
Cepheid AB was not required to submit a product dossier for Xpert HCV VL Fingerstick as per the *"Instructions for compilation of a product dossier"* (PQDx_018 version 3). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

Based on the product dossier screening and assessment findings, the product dossier for the Xpert HCV VL Fingerstick meets WHO prequalification requirements.

Manufacturing site inspection

At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine assessments of the Manufacturing sites will be conducted with copies of the WHO Public Inspection Reports (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current assessment performed at a manufacturing site for in vitro diagnostic products and summarises the assessment findings.

https://extranet.who.int/pqweb/vitro-diagnostics/who-public-inspection-reports

All published WHOPIRs are with the agreement of the manufacturer.

Based on the site inspection and corrective action plan review, the quality management system for Xpert HCV VL Fingerstick meets WHO prequalification requirements.

Product performance evaluation

The analytical performance evaluation of Xpert HCV VL Fingerstick took place at the National Serology Reference Laboratory (NRL), Melbourne, Australia, on behalf of WHO in the Q2 and Q3 of 2021, according to protocol PQDx_225, version 4.0.

Due to delays associated with the Covid-19 pandemic, the clinical part of the evaluation was considered as a commitment to prequalification (see below). The clinical evaluation was conducted at NRL from Q2 2022 to Q2 2023.

Clinical performance evaluation

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 116 fresh venous whole blood specimens was used. The specimens were characterised using the following comparator assay: cobas HCV Quantitative nucleic acid test for use on the cobas 6800/8800 Systems, Roche Molecular Systems, Inc..

Clinical performance characteristics in comparison with an agreed reference standard	
Sensitivity % (95% CI) (N=16)	100% (79.4% - 100%)
Specificity % (95% CI) (N=100)	100% (96.4% - 100%)
Invalid rate % (N=116)	0.9%
Bias	-0.441 $\log_{10}$ IU/mL

Limits of agreement	-0.887 to 0.005 log_{10} IU/mL

Note: The target sample size of 100 HCV-positive fresh whole blood specimens could not be reached within timeline, as only 16 HCV-positive specimens could be collected over a period of one year. Although an important negative bias was found compared to the comparator assay, the results were considered acceptable based on adequate sensitivity and specificity results, sample size limitations and moderate expected impact of this bias on clinical management.

Analytical performance evaluation

Analytical performance characteristics	
Limit of detection (LoD) (6^{th} WHO International Standard for HCV RNA Virus)	The LoD was estimated at 59.9 IU/mL (95% CI: 30.1 - 119.2). Although the LoD claimed by the manufacturer (22 IU/mL, 95% CI: 17-27) was not strictly verified, the estimated LoD was considered acceptable.
Within-run precision (repeatability)	At $3x10^2$ IU/mL, CV% were ≤5.6% (≤36.1% for lognormal CV) At 10^4 IU/mL, CV% were ≤2.0% (≤20.5% for lognormal CV)
Within-laboratory precision (reproducibility)	At $3x10^2$ IU/mL, CV% were ≤5.6% (≤36.1% for lognormal CV) At 10^4 IU/mL, CV% were ≤2.4% (≤24.1% for lognormal CV)
Genotype detection (4^{th} HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques, NIBSC code 14/290 and NRL HCV Mixed Genotype panel)	6 of the 7 specimens of the NIBSC panel were detected. Genotype 6I was not detected, which may be due to the fact that the panel was diluted 5-fold into whole blood. All 15 specimens (including genotype 6) of the NRL panel were detected.
Cross-contamination / carry-over	No carry-over was observed when high positive and negative specimens were tested alternatively.

Operational characteristics and ease of use

This assay requires laboratory equipment. It can be performed in laboratories with limited facilities; however, the instrument requires a stable source of electricity and a temperature-controlled and dust-free environment.

The assay was found easy to use by the operators performing the evaluation, who received a 0.5-day training from the manufacturer. It was noted that accurate specimen volume was required to be added to the cartridge to avoid Error results.

Key operational characteristics	
Validated specimen type(s) and volume	Capillary and venous EDTA whole blood (100 µL)
Number of steps for one specimen*	5 steps in total 1 step with precision pipetting if using venous whole blood (for capillary whole blood, use Minivette POCT)
Number of steps for instrument management**	4 steps per day
Time to result for one test	1 hour 15 minutes
Operator hands-on time for one test	15 minutes
Level of automation	Fully automated
Quality controls	Kit controls are not provided by the manufacturer. Two internal controls are included in each test.
Operating temperature	15-30 °C
Result display and connectivity	Results are displayed on the connected computer. They may be printed using a standard printer. The results can be exported to the laboratory information system and other health information systems.
Power sources	Main power Stable electricity is required
Biosafety *(outside of infectious specimen handling)*	Operators reported no biosafety concerns for the user outside of infectious specimen handling.
Waste	The volume of liquid was <10 mL per test. The volume of solid waste is approximately 45 g per test. Waste disposal may require specific measures in addition to usual laboratory biohazard waste disposal procedures. Cartridges may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures.

Calibration	No calibration is required
Maintenance	Daily, weekly, monthly and yearly maintenance is required.

** Steps for one specimen: each action required to obtain a result for one specimen (excluding specimen collection, instrument management, maintenance/calibration), e.g. add specimen to the cartridge, close the cartridge, scan/type specimen ID, load the cartridge on the instrument, press start (5 steps) OR scan/type specimen ID, load the specimen collection tube into the instrument, press start (3 steps)*

*** Steps for instrument management: each action required daily or per run to set up and shut down the instrument, e. g., switch on the instrument, login, maintain supplies, maintain reagents, discard liquid waste, discard solid waste, archive results, switch off the instrument (8 steps)*

Based on these results, the analytical performance evaluation for Xpert HCV VL Fingerstick meets the WHO prequalification requirements.

Labelling

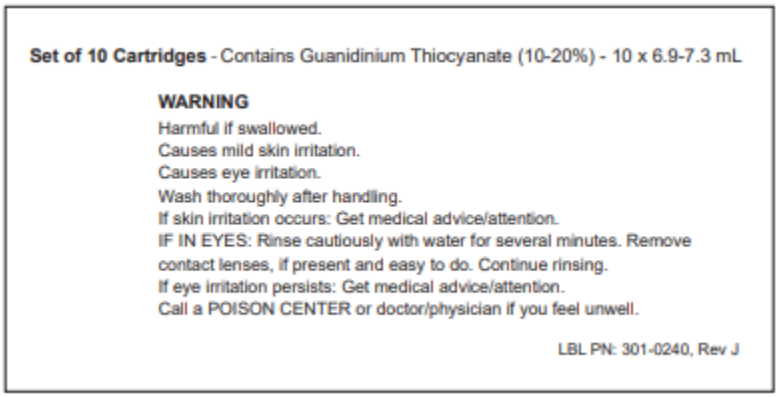
1. **Labels**
2. **Instructions for use**

1. Labels

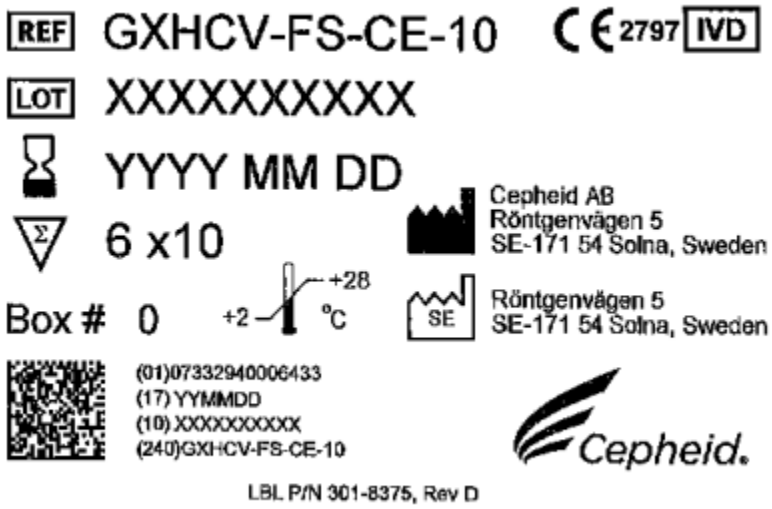
1.1 Carton/Box Top Label (P/N 301-8373)

1.2 Cartridge Label (P/N 301-8372)

1.3 Hazard Label (P/N 301-0240)

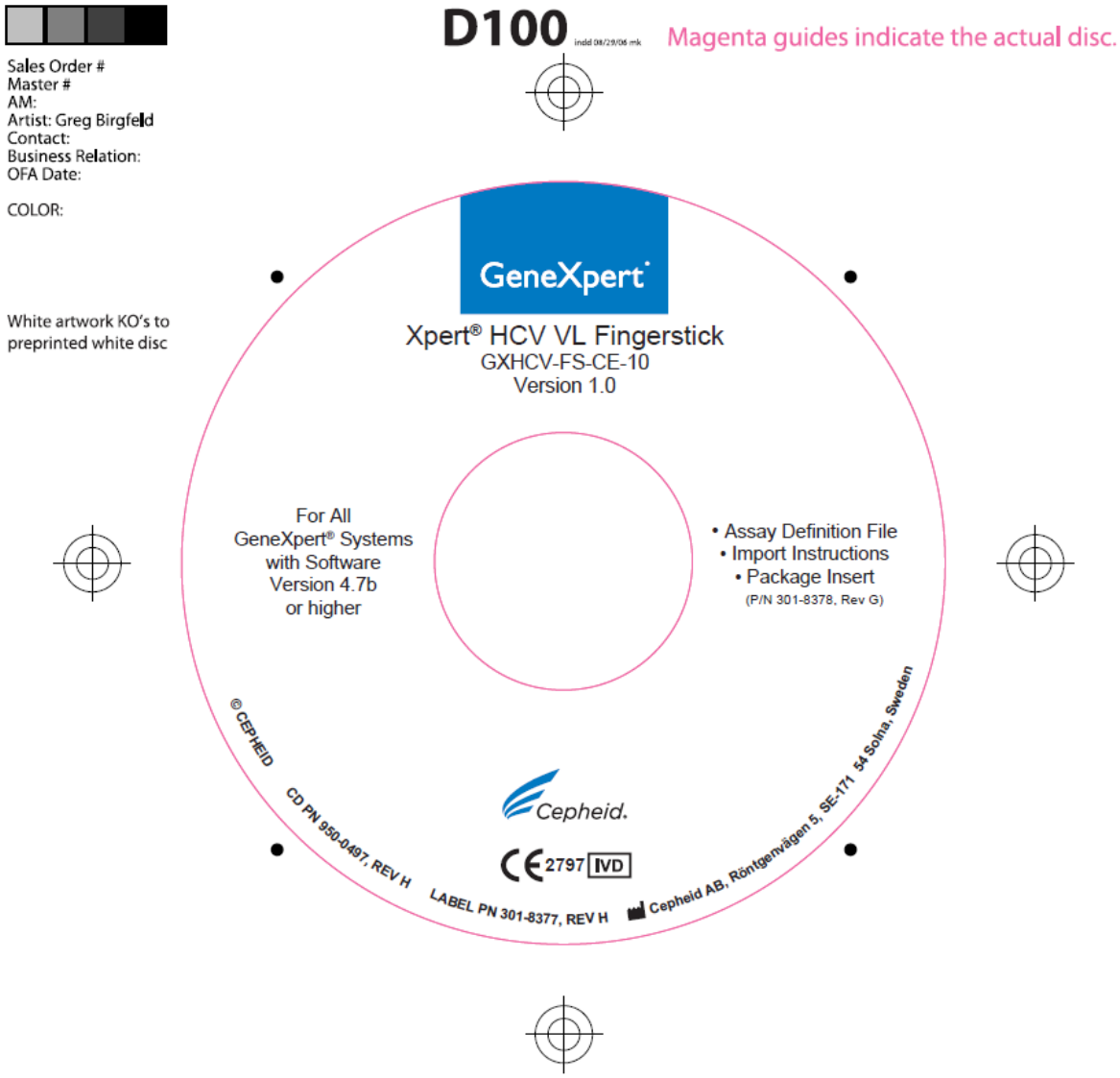


1.4 Shipper Label (P/N 301-8375)



1.5 CD Label (P/N 301-8377)

A label is printed onto the CD provided in the Xpert HCV VL Fingerstick kit.



2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® HCV VL Fingerstick

REF GXHCV-FS-CE-10

Cepheid®

In Vitro Diagnostic Medical Device

CE 2797 IVD

301-8378, Rev. G May 2021

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Cepheid AB
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Sweden

Xpert® HCV VL Fingerstick

For In Vitro Diagnostic Use Only.

1 Proprietary Name

Xpert® HCV VL Fingerstick

2 Common or Usual Name

HCV VL FS

3 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 FS 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 FS 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.

The Xpert HCV VL FS 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.

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, unsafe health care injections, and receipt of unscreened donated blood products. Less frequently, HCV has been shown to be transmitted through perinatal and sexual exposures.[2] HCV is usually asymptomatic during the acute phase of the infection and thus many people go undiagnosed. Of those infected with HCV approximately 70% will go on to develop chronic HCV disease.[2] Presently, screening for past or present HCV infection is based on the detection of HCV antibodies, however, the presence of HCV RNA is indicative of current infection.[3] An estimated 71 million people worldwide live with chronic HCV and only 20% have been diagnosed.[2] HCV infection is unevenly distributed worldwide and prevalence varies across and within countries. The regions most affected are the Eastern Mediterranean (2.3%), Europe (1.5%), Africa (1.0%) and <1% in other regions such as the Americas, Western Pacific and South-East Asia.[2] Antiviral medicines can cure HCV, but access to diagnosis and treatment is low.[4] A cure for HCV infection (defined as sustained virologic response, i.e. undetectable HCV RNA 12 or 24 weeks after the completion of HCV therapy) is now possible in most patients with highly effective, safe and tolerable combinations of oral direct-acting antivirals (DAAs) taken for 8–12 weeks.[2,5]

Quantitation of HCV RNA has proven useful 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, and at 12 or 24 weeks post completion of HCV therapy.[5]

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 reverse transcription PCR (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, see the appropriate *GeneXpert Dx Operator Manual* or *GeneXpert Infinity Operator Manual*. The

HCV VL FS assay includes reagents for the detection of HCV RNA in clinical specimens as well as two internal controls used for quantitation of HCV RNA. The internal controls are also used to control for adequate processing of the target and to monitor 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.

The assay is standardized against the 4th World Health Organization (WHO) International Standard for HCV (NIBSC code: 06/102).[6]

6 Reagents and Instruments

6.1 Materials Provided

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

HCV VL FS 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)	1.0 mL per cartridge
• Rinse Reagent	0.5 mL per cartridge
• Elution Reagent	1.5 mL per cartridge
• Binding Reagent	1.5 mL per cartridge

CD

- 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.cepheid.com and 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 FS assay cartridges and reagents at 2–28 °C. Bring the cartridges to room temperature prior to use if they have been stored cold.
- Do not open the cartridge lid until you are ready to perform the assay.
- Do not use a cartridge that has leaked.
- Do not use cartridges that have been previously frozen.
- Do not use a cartridge past the expiration date.

8 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert 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
- Ethanol or denatured ethanol
- Disposable Minivette® POCT 100 µL K3E (P/N: MINIVETTE 100E-100, 100 per box)
- Disposable Safety-Lancet Super, 1.5 mm (Sarstedt P/N: 85.1018) or similar

9 Warnings and Precautions

- For *In Vitro* Diagnostic Use Only.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[7] and the Clinical and Laboratory Standards Institute.[8]
- 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 FS assay reagents with other reagents.
- Do not open the HCV VL FS 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 FS assay cartridge is used to process one test. Do not reuse spent cartridges.
- The Minivette POCT is used to collect and transfer one specimen. Do not reuse spent Minivette POCT.
- 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 freshly prepared solution of 0.5% sodium hypochlorite (or a 1:10 dilution of household chlorine bleach). Follow by wiping the surface with 70% ethanol. Let work surfaces dry completely before proceeding.
- 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.[9]

10 Chemical Hazards[10,11]

- 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 or 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 and Storage

11.1 Capillary Whole Blood

- Capillary whole blood specimens should be collected using the Minivette POCT subsequent to puncture of the finger with the Safety Lancet (not provided by Cepheid). Follow the instructions for use per the manufacturer.
- 100 µL whole blood is required for the HCV VL FS assay. The Minivette POCT must be filled completely.
- Whole blood specimens collected with the Minivette POCT may be held in the collection device for up to 15 minutes at 5 – 35°C.

11.2 Venous Whole Blood

- Collect venous whole blood in a sterile vial using EDTA as the anticoagulant as per manufacturer's instructions for use.
- 100 µL whole blood is required for the HCV VL FS assay.
- Venous whole blood collected in EDTA may be stored in a sterile vial for up to 6 months at -20 °C, 72 hours at 2 – 8 °C or 24 hours at a maximum of 35 °C.

12 Procedure

12.1 Preparing the Cartridge

Important **Start the test within four hours of adding the sample to the cartridge.**

1. Wear protective disposable gloves.
2. Allow the cartridge to adjust to room temperature (25±3°C) prior to use.
3. Inspect the cartridge for damage. If damaged, do not use it.
4. Label the cartridge with specimen identification.
5. Open the lid of the cartridge.
6. For *capillary* whole blood:
   A. Place the filled Minivette POCT (see Figure 1) as deep as possible in the sample chamber of the cartridge (see Figure 2).
   B. Gently press down the piston of the Minivette POCT to dispense the blood.

   For *venous* whole blood:
   A. Mix the whole blood by inverting the vial at least seven (7) times.
   B. Use a micropipette to transfer 100 µL of whole blood into the sample chamber of the cartridge (see Figure 2). Ensure that the blood is dispensed into the bottom of the sample chamber.
   C. To ensure that 100 µL is dispensed, use a pre-wet pipette tip or use the reverse pipetting technique to aspirate and dispense the blood sample.

Note Loading less than 100 µL blood into the cartridge may trigger an insufficient volume error (ERROR 2097), preventing the instrument from running the sample.

Figure 1. Minivette POCT 100 µl EDTA

Note Do not remove the thin plastic film that covers the inner ring of 13 ports of the test cartridge.

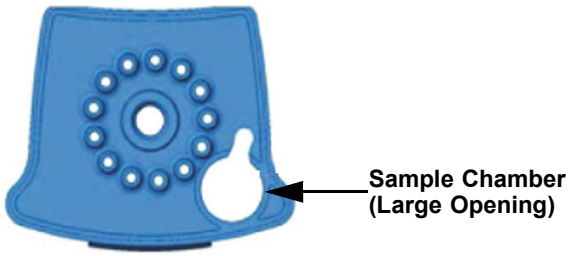

Figure 2. HCV VL FS Cartridge (Top View)

7. Close the cartridge lid. Ensure the lid snaps firmly into place.

12.2 Starting the Test

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

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 of instrument that is being used.

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

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 Dx software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. The Xpertise™ Software will launch automatically. If it does not, 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 FS cartridge. 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. If using a GeneXpert Infinity instrument, 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

   If using a GeneXpert Dx Instrument:

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

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

   F. Wait until the instrument releases the door lock before opening the module door and removing the cartridge.

   G. 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) control, Internal Quantitative Standard High and Low (IQS-H and IQS-L), and a Probe Check Control (PCC).

- **Sample Volume Adequacy (SVA)**: Ensures that 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 2097** will display if an insufficient quantity of sample has been added to the cartridge. **ERROR 2096** indicates that not enough sample has been processed. 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® controls unrelated to HCV in the form of a dry bead that goes through the whole GX process. They are used to equilibrate the HCV concentration with the quality of the specimen and the characteristics of the kit lot. IQS-H and IQS-L detect specimen-associated inhibition of the RT-PCR reaction, thereby, acting as sample processing controls. The IQS-H and IQS-L pass if they meet the validated acceptance criteria.
- **Lot Specific Parameters (LSP)** for quantification: Each kit lot has built-in LSP generated from an HCV calibration panel traceable to the 4th WHO International Standard for HCV NAT (NIBSC code 06/102)[7] **and the internal quantitative standards, IQS-H and IQS-L**. The Ct values of the IQS-H and IQS-L are included as parameters in the equation that forms the LSP of the kit lot.
- **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 the fluorescence signals meet the validated acceptance criteria.
- **External Controls**: Following good laboratory practice, external controls, not provided in the kit, should be used in accordance with the requirements of local and state accrediting organizations 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 shown in the View Results window (Figure 3 and Figure 4). Possible results are shown in Table 1.

Table 1. HCV VL FS Assay Results and Interpretation

Result	Interpretation
HCV DETECTED **XX IU/mL (log X.XX)**	HCV RNA is detected at XX IU/mL (see Figure 3). • The HCV RNA has a titer within the quantitative range of the assay (100-1.00E08 IU/mL). • IQS-H and IQS-L: PASS. • Probe Check: PASS; all probe check results pass.
HCV DETECTED **> 1.00E08 IU/mL**	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 **< 100 IU/mL**	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	HCV RNA is not detected (see Figure 4). • IQS-H and IQS-L: PASS. • Probe Check: PASS; all probe check results pass.

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

Result	Interpretation
INVALID	Presence or absence of 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. • Probe Check: PASS; all probe check results pass.
ERROR	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 valid 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 screen shots are for example only. The version number may vary from the screen shots shown in this package insert.

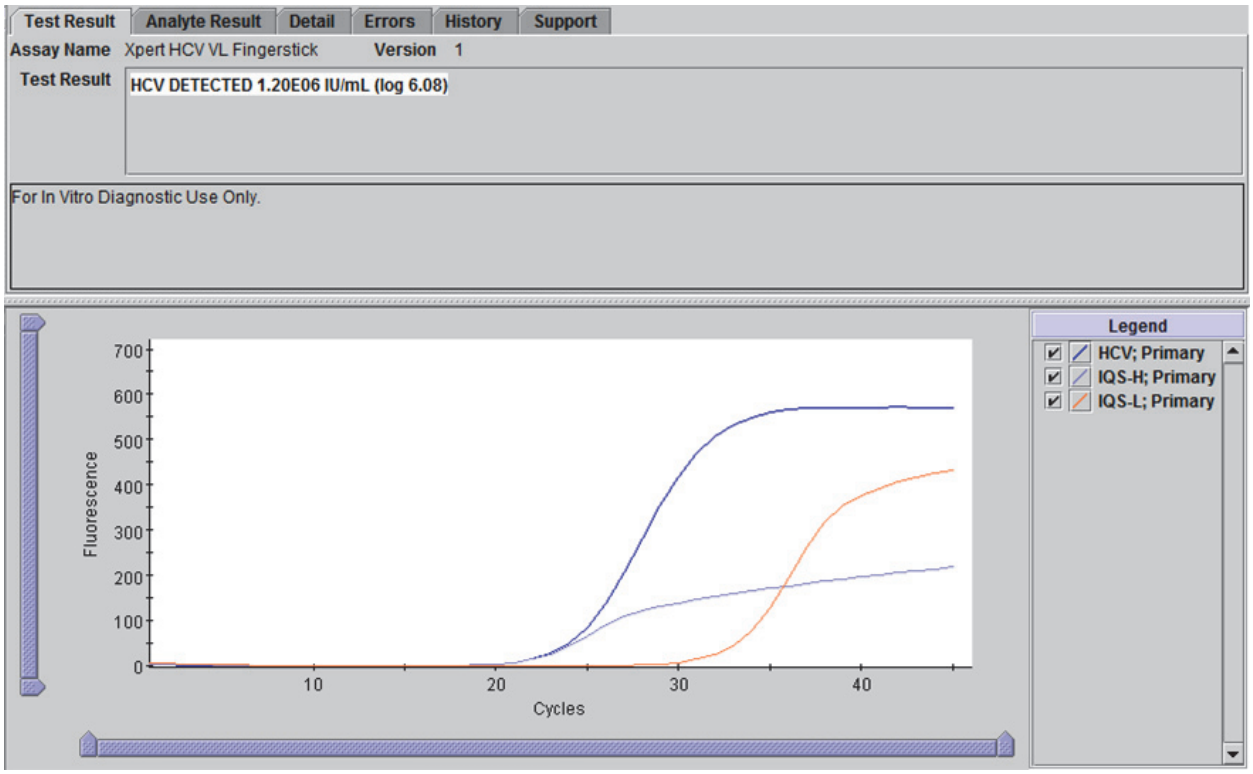

Figure 3. HCV Detected and Quantified

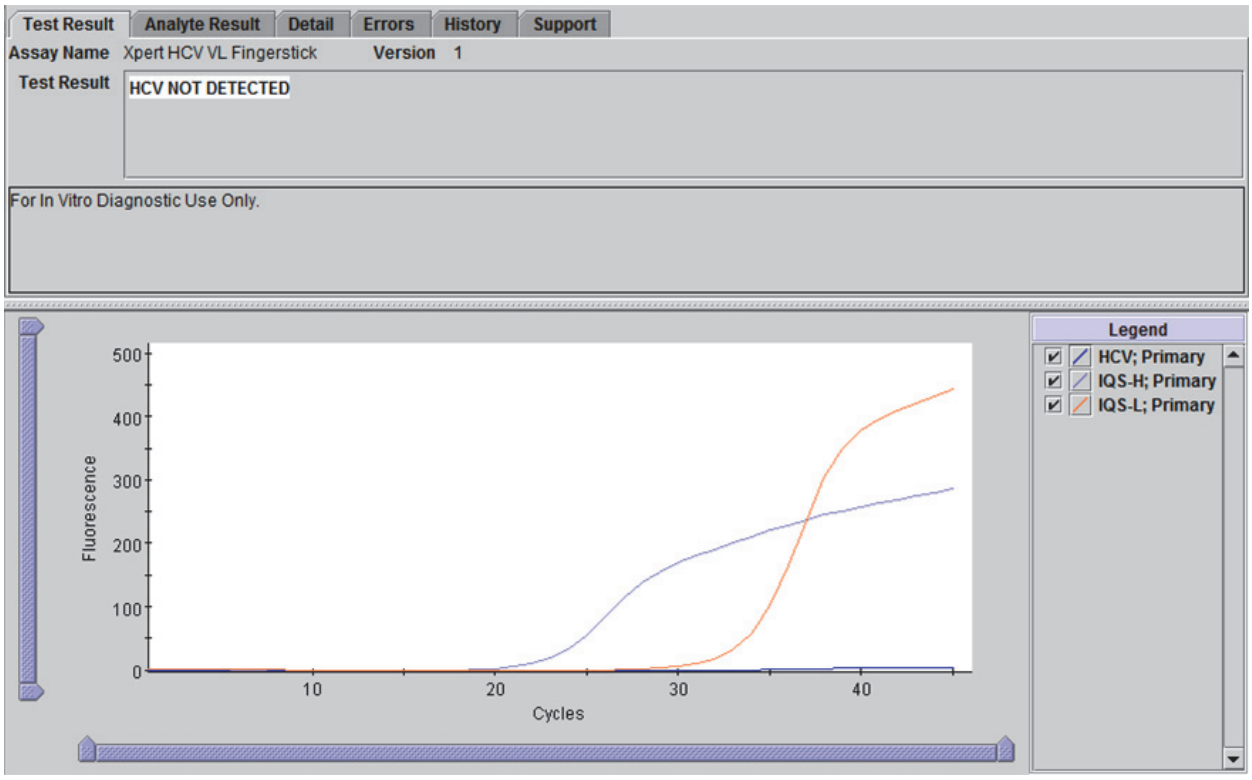

Figure 4. HCV Not Detected

16 Retests

16.1 Reasons to Repeat the Test

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 the 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

If the result of a test is either **INVALID**, **ERROR**, or **NO RESULT**, use a new cartridge to retest the affected specimen (do not re-use the cartridge).

1. If using capillary whole blood, see Section 11.1, Capillary Whole Blood for the collection of a specimen.
2. Remove a new cartridge from the kit.
3. See Section 12, Procedure, including Section 12.1, Preparing the Cartridge, and Section 12.2, Starting the Test.

17 Procedural Limitations

- Good laboratory practices and changing gloves between the handling of specimens are recommended to avoid contamination of specimens or reagents.
- Rare mutations within the target region of the HCV VL FS assay may affect primer or probe binding resulting in under-quantitation or failure to detect the virus.
- This assay has been validated only for use with capillary and venous whole blood collected in EDTA. Testing of other specimen types may result in inaccurate results.
- Proper performance of this test requires appropriate specimen collection, storage, handling, and transport to the test site.
- Results from the HCV VL FS assay should be interpreted in conjunction with other clinical and laboratory findings.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to quantify technology differences.
- A negative test result with the HCV VL FS assay does not preclude a patient from having an HCV infection.
- The assay is not for the screening of blood, plasma, serum, or tissue donations for HCV.

18 Performance Characteristics

18.1 Limit of Detection

The limit of detection (LOD) of the HCV VL FS assay was determined for HCV genotypes 1 through 6 by testing seven or eight member panels prepared by spiking HCV-positive clinical samples or a commercial high-titer HCV reference material (Acrometrix HCV High Titer Control, genotype 1b, Thermo Fisher Scientific, Lot: RD16121511) into HCV-negative human EDTA whole blood. The concentration of each stock was determined by the Xpert HCV Viral Load assay, with calibration traceable to the 4th WHO International Standard for HCV (NIBSC code 06/102).[6] The LOD of HCV genotype 1a was determined by testing 24 replicates of each dilution level for each of three reagent lots over three days. In total 72 replicates per level of genotype 1a were tested. The LOD for HCV genotypes 1b and 2 through 6 was determined by using one reagent lot to test a total of 24 replicates of each dilution level over three days.

The HCV RNA concentration that can be detected with a positivity rate of 95% was determined by PROBIT regression analysis. The results for all genotypes are presented in Table 2.

Table 2. Limit of Detection for the Xpert HCV VL FS Assay using PROBIT Regression

Genotype	Nominal Concentration (IU/mL)	Number of Valid Replicates	Number of Positives	Positivity Rate (%)	LOD with 95% Probability Estimated by PROBIT (95% Confidence Interval)
1a	45	72	72	100%	22 IU/mL[a] (95% CI: 17 – 27 IU/mL)
	30	72	70	97%	
	15	72	59	82%	
	10	72	59	82%	
	5	72	30	42%	
	2.5	72	9	13%	
1b	45	24	24	100%	23 IU/mL (95% CI: 18 – 32 IU/mL)
	30	24	23	96%	
	15	24	19	79%	
	10	24	18	75%	
	5	24	13	54%	
	2.5	24	11	46%	

Table 2. Limit of Detection for the Xpert HCV VL FS Assay using PROBIT Regression (Continued)

Genotype	Nominal Concentration (IU/mL)	Number of Valid Replicates	Number of Positives	Positivity Rate (%)	LOD with 95% Probability Estimated by PROBIT (95% Confidence Interval)
2b	45	24	24	100%	13 IU/mL (95% CI:10 – 16 IU/mL)
	30	24	24	100%	
	15	24	24	100%	
	10	24	18	75%	
	5	24	12	50%	
	2.5	24	9	38%	
3a	45	24	24	100%	28 IU/mL (95% CI: 21 – 34 IU/mL
	30	24	22	92%	
	15	24	17	71%	
	10	24	14	58%	
	5	24	12	50%	
	2.5	24	4	17%	
4	45	24	24	100%	15 IU/mL (95% CI: 13 – 20 IU/mL)
	30	24	24	100%	
	15	24	20	83%	
	10	24	21	88%	
	5	24	16	67%	
	2.5	24	8	33%	
5	45	24	24	100%	28 IU/mL (95% CI: 21 – 36 IU/mL)
	30	23	21	91%	
	15	24	17	71%	
	10	24	15	63%	
	5	24	11	46%	
	2.5	24	9	38%	
6e	60	24	24	100%	35 IU/mL (95% CI: 28 – 42 IU/mL)
	45	24	23	96%	
	30	24	22	92%	
	15	24	14	58%	
	10	24	11	46%	
	5	24	10	42%	
	2.5	24	3	13%	

a. The highest estimated LOD for genotype 1a based on testing and analysis of each of three reagent lots.

18.2 Lower Limit of Quantitation

The lower limit of quantitation (LLOQ) is defined as the lowest concentration of HCV RNA that is quantified with acceptable precision and trueness and is determined using the total analytical error (TAE) and an approach based upon the difference between two measurements. The LLOQ was evaluated with four independent low titer samples tested using three reagent lots with 22 – 24 replicates per lot. TAE was estimated with the Westgard model according to the CLSI guideline EP17-A2[12] with the criterion, [(Absolute Bias) + 2 SDs ≤ 1 $\log_{10}$ IU/mL]. The difference between two measurements approach was evaluated with the criterion, [(2 × SQRT(2) × SD) ≤ 1 $\log_{10}$ IU/mL]. The LLOQ analyses for each sample are presented in Table 3. The results demonstrate that the HCV VL FS assay can quantify 100 IU/mL of HCV RNA with an acceptable trueness and precision.

Table 3. Determination of the LLOQ for the Xpert HCV VL FS Assay

Specimen	Lot	N	HCV Concentration (Log10 IU/mL)		Bias	Total SD	Total Analytical Error[a]	Two Measurement Approach[b]
			Expected	Observed				
HCV Gt1a (Clinical Specimen #1)	1	24	2.00	2.16	0.16	0.23	0.61	0.64
	2	24	2.00	2.13	0.13	0.20	0.53	0.56
	3	23	2.00	2.30	0.30	0.20	0.70	0.56
	1	24	1.65	1.70	0.04	0.30	0.64	0.85
	2	24	1.65	1.62	0.03	0.26	0.55	0.74
	3	24	1.65	1.77	0.12	0.18	0.48	0.51
HCV Gt1a (Clinical Specimen #2)	2	24	2.00	1.90	0.10	0.23	0.56	0.65
	3	22	2.00	2.11	0.11	0.27	0.65	0.76
	4	24	2.00	1.96	0.04	0.24	0.52	0.68
HCV Gt3a (Clinical Specimen #3)	1	24	1.65	1.52	0.13	0.27	0.66	0.75
	2	23	1.65	1.58	0.07	0.29	0.66	0.83
	3	23	1.65	1.64	0.02	0.25	0.52	0.71

a. TAE calculated according to the Westgard model where [TAE = (| Bias | + (2×SD)) ≤ 1 $\log_{10}$ IU/mL], ensuring there is a 95% probability that the measurement will be less than 1 $\log_{10}$ IU/mL from the true value.

b. Two measurements approach where [(2 × SQRT(2) × SD) ≤ 1 $\log_{10}$ IU/mL] indicates that a difference of less than 1 $\log_{10}$ IU/mL can be explained by a random measurement error

18.3 Linear Range and Inclusivity

The linearity of the HCV VL FS assay was determined for HCV genotypes 1 through 6 using sample panels prepared by spiking HCV positive clinical specimens or armored RNA in negative human EDTA whole blood. The concentrations of clinical specimen and armored RNA were determined using HCV RNA quantitative, CE-marked nucleic acid tests. Each panel member was tested in replicates of six except for the lowest level of each (50 IU/mL) which was tested in replicates of twelve. HCV genotypes 1 and 3 were tested using two reagent lots whereas the other HCV genotypes (2, 4, 5 and 6) were tested using one reagent lot.

The linearity was demonstrated for all genotypes according to CLSI guideline EP06-A[13]. The results for HCV genotypes 1 through 6 are shown in Figure 5, with pooled results shown for HCV genotypes 1 and 3.

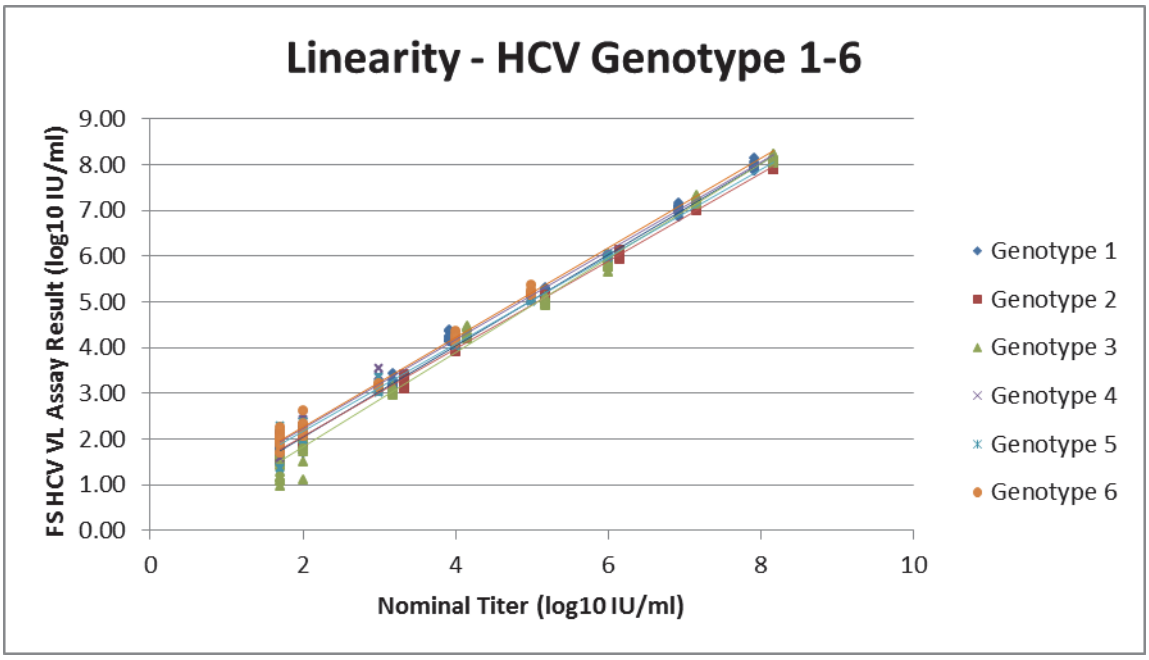

Figure 5. Linearity for the Xpert HCV VL FS Assay

The HCV VL FS assay is linear across a range of 100 – 1 x 10^8 IU/mL with a $R^2 > 0.99$ for HCV genotypes 1 through 3 and across the dynamic range tested for HCV genotypes 4 through 6 with an $R^2 > 0.98$ (Table 4).

Table 4. Linear Regression of Xpert HCV VL FS assay with Tested Titer Range per Genotype

Genotype	Linear Regression Equation	R^2	Tested Titer Range log_{10} IU/ml
1	y = 0.9975x + 0.0603	0.995	1.70 - 8.00
2	y = 0.9564x + 0.1547	0.997	1.70 - 8.00
3	y = 1.0312x - 0.2348	0.993	1.70 - 8.00
4	y = 0.9683x + 0.3056	0.986	1.70 - 5.00
5	y = 0.9553x + 0.2645	0.990	1.70 - 6.00
6	y = 0.9798x + 0.2995	0.989	1.70 - 5.00

18.4 Precision/Reproducibility

The precision/reproducibility of the HCV VL FS assay was evaluated in EDTA whole blood using an analysis of variance (ANOVA) to estimate total variance.

The study was a multi-center (3 sites; 2 external and 1 internal) blinded study to estimate the major components of variance of the HCV VL FS assay using an eight-member panel consisting of seven HCV positive members and a HCV negative EDTA whole blood sample. Low titer members were prepared using a well characterized HCV genotype 1 sample whereas higher titer members were prepared using an armored RNA HCV genotype 1 stock. Two operators, one with prior PCR experience and one without, at each of the three study sites, tested one panel in triplicate two times per day (equivalent to twelve replicates per day) over six testing days. Three lots of the HCV VL FS assay were used, with each lot representing two days of testing. A total of 216 replicates of each panel member were tested. Precision and reproducibility were evaluated in accordance with CLSI EP5-A3[14] and CLSI EP15-A3[15].

The precision and reproducibility of the HCV VL FS 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 log10 HCV transformed concentrations were calculated as shown in Table 5.

Table 5. Precision/Reproducibility of the Xpert HCV VL FS Assay

HCV RNA Concentration (log10 IU/mL)			Contribution to Total Variance SD (CV%)										Total Precision	
			Site/Inst		Lot		Day		Operator/ Run		Within-run			
Expected	Actual	N	SD	(%)a	SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	CV[b]
8.00	7.73	216	0.09	30.2	0.06	16.5	<0.01	<0.1	0.04	7.2	0.11	46.1	0.15	36.8
7.00	6.77	216	0.08	36.8	0.05	15.5	0.04	8.7	0.03	5.1	0.08	33.9	0.14	32.8
5.70	5.68	216	<0.01	<0.1	0.06	27.0	0.02	5.3	0.01	0.7	0.09	66.9	0.11	25.1
4.00	3.88	216	0.09	35.9	0.04	5.9	<0.01	<0.1	0.01	0.8	0.11	57.3	0.15	35.1
3.00	3.00	213[c]	0.04	11.8	<0.01	<0. 1	0.02	3.5	0.01	1.0	0.10	83.7	0.11	26.4
2.00	1.97	216	0.03	2.2	<0.01	<0.1	0.03	2.5	<0.01	<0.1	0.20	95.3	0.20	49.2

a. (%) is contribution of variance component to overall variance
b. "CV" is lognormal CV, as obtained using the formula: Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100
CV(%) = percent coefficient of variation; SD = standard deviation; sqrt = square root
c. Three samples with one as **ERROR**, one as **INVALID**, the third one as **HCV NOT DETECTED** are excluded.

Table 6 shows the positive percent agreement (PPA) and negative percent agreement (NPA) for a panel member targeting an HCV RNA concentration below the limit of quantitation (i.e., 1.60 $\log_{10}$ IU/mL or 40 IU/mL) and an HCV negative EDTA whole blood panel member.

Table 6. Positive and Negative Percent Agreement for Panel Member Below the Limit of Quantitation

Expected HCV RNA Concentration	Number of Tests with Valid Results	Positive Results	Negative Results	Positive Percent Agreement[a]	Negative Percent Agreement[b]	95% CI[c]
1.60 Log_{10} IU/mL	215	214	1	99.5		(97.4, 99.9)
Negative	216	1	215		99.5	(97.4, 99.9)

a. Positive Percent Agreement = (number of positive results/total number of valid tests in positive panel member) × 100
b. Negative Percent Agreement = (number of negative results/total number of valid tests in negative panel member) × 100
c. Confidence interval calculated using the Wilson-Score method

18.5 Analytical Specificity (Exclusivity)

The analytical specificity of the HCV VL FS assay was evaluated by adding potentially cross-reacting organisms at 1×10^5 CFU/mL, copies/mL or TCID_{50}/mL input concentration into HCV negative EDTA whole blood and into EDTA whole blood that contained 300 IU/mL HCV reference material (HCV genotype 1b calibrated against the WHO 4th International standard, NIBSC code 06/102)[6]. Tested organisms are listed in Table 7. None of the tested organisms showed cross reactivity or interfered with the quantification of the HCV VL FS assay.

Table 7. Analytical Specificity Organisms

Viruses		Bacteria	Yeast
Banzi virus	Human papilloma virus 16	*Staphylococcus epidermidis*	*Candida albicans*
BK Human polyoma virus	Human papilloma virus 18	*Staphylococcus aureus*	
Cytomegalovirus	Human T-cell lymphotropic virus types 1 and 2		
Dengue virus	St. Louis Encephalitis virus		
Epstein-Barr virus	Varicella Zoster virus		
Hepatitis A virus	Vaccina virus		

Table 7. Analytical Specificity Organisms (Continued)

Viruses		Bacteria	Yeast
Hepatitis B virus	Ilheus virus		
Herpes simplex virus 1	West Nile virus		
Herpes simplex virus 2	Yellow Fever virus		
Human herpes virus 6	Zika virus		
Human herpes virus 8			
Human Immunodeficiency Virus-1			
Human Immunodeficiency Virus-2			

18.6 Potentially Interfering Substances

The susceptibility of the HCV VL FS assay to interference by elevated levels of endogenous substances, by autoimmune disease markers, and by drugs prescribed to HCV infected patients was evaluated. The inhibitory effects were evaluated both in the presence and absence of 300 IU/mL HCV RNA reference material (HCV genotype 1b, calibrated against the WHO 4th International standard, NIBSC code 06/102).[6]

Elevated levels of the endogenous substances listed in Table 8 were shown not to interfere with the quantification of the HCV VL FS assay or impact the assay specificity.

Table 8. 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 drugs as presented in Table 9 were shown to not interfere with the quantification of the HCV VL FS assay or impact the assay specificity when tested at three times peak level concentration in five drug pools.

Table 9. Drug Pools Tested

Pool	Drugs
1	Zidovudine, Abacavir sulfate, Saquinavir, Ritonavir, Interferon-alfa 2b, Ombitasvir, Paritaprevir, Dasabuvir
2	Fosamprenavir, Ribavirin, Ledipsavir, Sofosbuvir, Daclatasvir, Simeprevir, Peginterferon-alfa 2a, Peginterferon-alfa 2b
3	Tenofovir disoproxil fumarate, Lamivudine, Indinavir sulfate, Ganciclovir, Acyclovir, Valganciclovir HCl
4	Stavudine, Efavireniz, Lopinavir, Enfuvirtide, Ciprofloxacin, Clarithromycin, Maraviroc
5	Nevirapine, Nelfinavir, Azithromycin, Valacyclovir

Testing of specimens from twelve individuals with autoimmune disorders which tested positive for the systemic lupus erythematosus (SLE) marker, of which seven were also anti-nuclear antibody (ANA) positive, and testing of specimens from eight individuals testing positive for rheumatoid factor (RF) showed no interference with either the quantification or specificity of the HCV VL FS assay.

18.7 Seroconversion Sensitivity

The sensitivity of the HCV VL FS assay was evaluated by testing sequential plasma specimens from ten seroconversion panels. As the HCV VL FS assay uses EDTA whole blood as the specimen type, each plasma specimen was diluted in EDTA whole blood before testing (1:3 dilution). The HCV VL FS assay detected HCV RNA in 53 out of 59 specimens as compared to 22 out of 59 specimens 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). A positive HCV test result was generated earlier with the HCV VL FS assay in all ten panels tested as compared to the HCV antibody screening. The seroconversion sensitivity is presented in Table 10.

Table 10. Seroconversion Sensitivity of the HCV VL FS Assay

Panel No.	Number of Specimens in Panel	Days Spanned	Number of Reactive Panel Members		Days to First Reactive Result		Titer at first reactive result with HCV VL FS (IU/mL)[a]	Days between first reactive result with Xpert and any Ab test
			HCV VL FS	Antibody Test[b]	HCV VL FS	Antibody Test[b]		
PHV913	4	9	4	2	0[c]	7	1.18E+03	7
PHV915	4	14	4	2	0[c]	12	5.10E+01	12
PHV920M	10	35	10	9	0[c]	7	1.19E+06	7
PHV922	6	17	6	5	0[c]	3	1.57E+06	3
PHV924	6	88	6	3	0[c]	59	3.81E+06	59
PHV925	5	27	5	1	0[c]	27	1.33E+06	27
PHV926	5	14	5	1	0[c]	14	1.13E+05	14
PHV927	5	17	4	0	4	17[d]	6.66E+02	13
PHV928	9	50	7	0	29	50[d]	5.40E+01	21
PHV929	6	22	3	0	14	22[d]	2.36E+03	8

a. Titer from raw data has been multiplied by a factor of three (3) to compensate for dilution in whole blood.
b. Antibody test based on vendor data: Abbot ARCHITECT HCV Ab, Abbot PRISM HCV Ab, Ortho Version 3.0 ELISA HCV Ab, Ortho HCV 3.0 ELISA Test System with Enhanced SAVe, Ortho Vitros ECi, Siemens ADVIDA Centaur.
c. All bleeds were detected with the HCV VL FS assay.
d. All bleeds were non-reactive for HCV antibodies (based on vendor information). The last bleed day is used to determine “Days to First Reactive Result.”

19 Performance Characteristics – Clinical Performance

19.1 Specificity in Normal Healthy Blood Donors

The specificity of the Xpert HCV VL FS assay was evaluated using 500 EDTA whole blood specimens from HCV negative blood donors. HCV RNA was not detected in any of the 500 specimens tested by the Xpert HCV VL FS assay demonstrating 100% specificity (95% CI = 99.2-100). The indeterminate rate for the Xpert HCV VL FS assay in normal human blood donors was 1.0% (5/505).

19.2 Clinical Performance

A multi-site study was conducted to evaluate the performance of the Xpert HCV VL FS assay with capillary and venous whole blood specimens from individuals at high risk for HCV infection and individuals infected with HCV, relative to an HCV RNA quantitative comparator method in EDTA plasma.

Of the 930 eligible subjects, 621 (66.8%) were male, and 309 (33.2%) were female. The average age was 48.8 ± 12.6 years with an age range of 18 to 84 years.

Performance in a High Risk Population

The sensitivity and specificity of the Xpert HCV VL FS assay was assessed using specimens collected from individuals determined to be at risk for HCV infection. Table 11 shows the performance of the Xpert HCV VL FS assay using capillary and venous WB specimens, relative to an HCV RNA quantitative comparator method using EDTA plasma from the same specimen. The table also shows the performance of the Xpert HCV VL FS assay using capillary and venous WB specimens as compared to the Xpert HCV VL assay with EDTA plasma from the same specimen.

Table 11. Performance of the Xpert HCV VL FS Assay versus Two HCV RNA Comparator Methods in a HCV High Risk Population

	N	TP[a]	FP[b]	TN[c]	FN[d]	Sensitivity (%)	95% CI of Sensitivity (%)	Specificity (%)	95% CI of Specificity (%)
HCV VL FS Capillary vs Comparator (Plasma)	339	54	0	283	2	96.4	87.9 – 99.0	100	98.7 – 100
HCV VL FS Venous vs Comparator (Plasma)	352	55	0	295	2	96.5	88.1 – 99.0	100	98.7 – 100
HCV VL FS Capillary vs HCV VL (Plasma)	339	54	0	278	7	88.5	78.2 – 94.3	100	98.6 – 100
HCV VL FS Venous vs HCV VL (Plasma)	352	55	0	290	7	88.7	78.5 – 94.4	100	98.7 – 100

a. True Positive
b. False Positive
c. True Negative
d. False Negative

Method Correlation

Of the specimens from 930 total subjects, 881 had valid test results for both Xpert HCV VL FS assay using capillary whole blood and the HCV RNA comparator method with an overall agreement of 97.8% (862/881). Of the 881, 429 were within the quantitation range of both assays. The result of the Deming regression analysis is shown below in Figure 6.

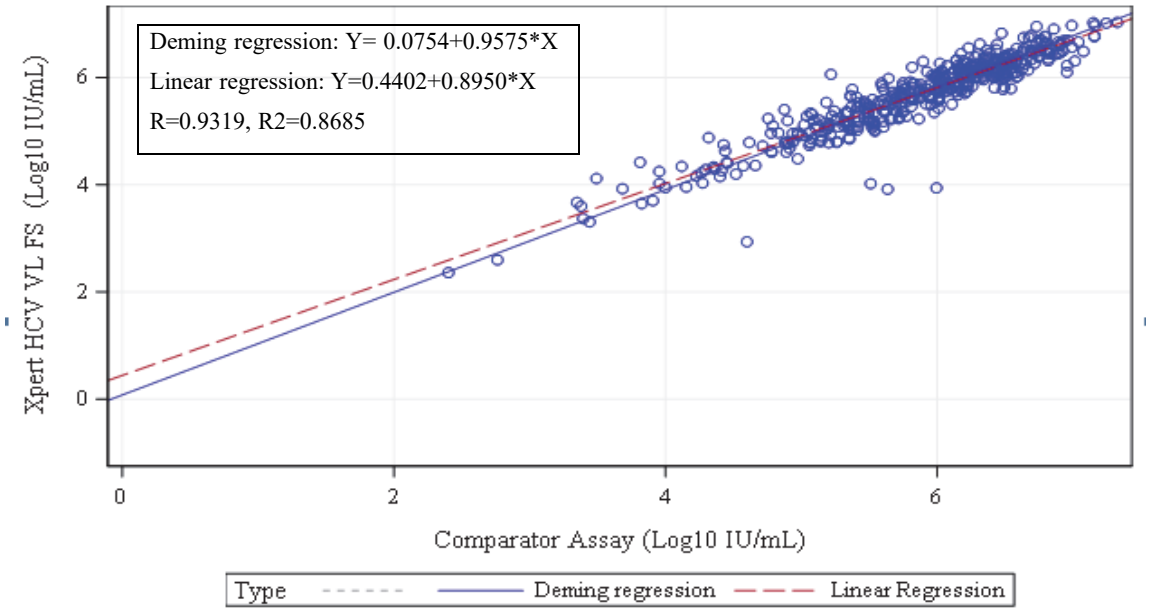

Figure 6. Xpert HCV VL FS Assay (Capillary WB) vs HCV RNA Comparator Method (EDTA Plasma)

Of the specimens from 930 total subjects, 920 had valid test results for both Xpert HCV VL FS assay in venous whole blood and comparator method with an overall agreement of 97.7% (899/920). Of the 920, 447 were within the quantitation range of both assays. The result of the Deming regression analysis is shown below in Figure 7.

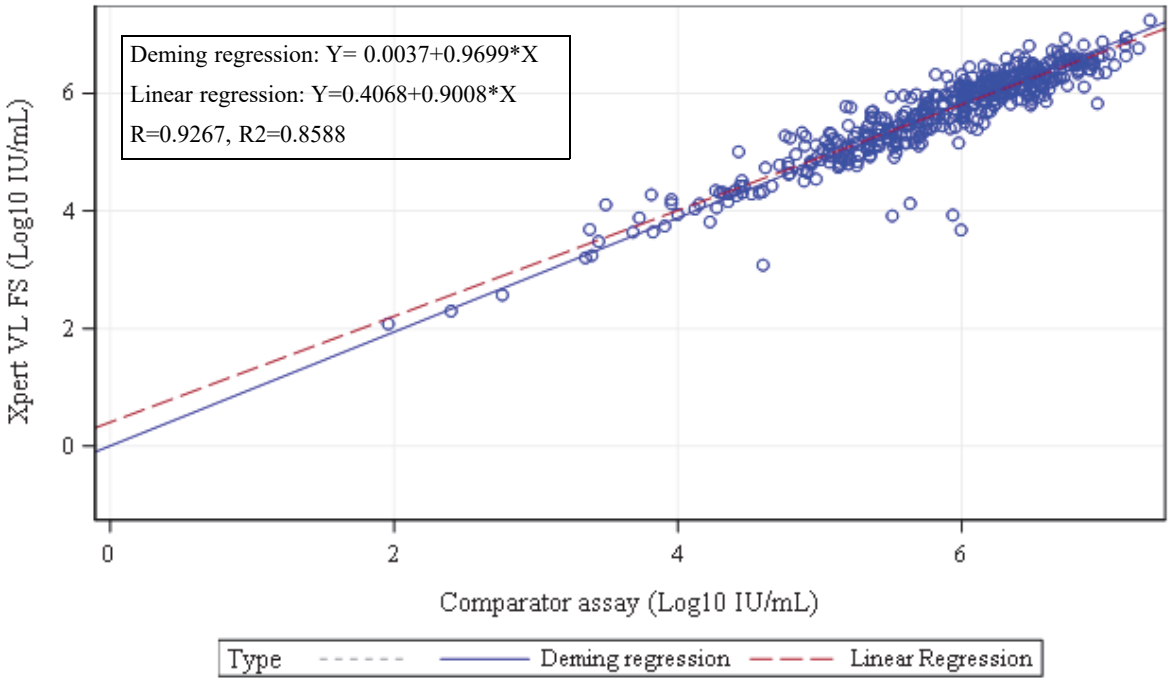

Figure 7. Xpert HCV VL FS Assay (Venous WB) vs HCV RNA Comparator Method (EDTA Plasma)

Of the specimens from 930 total subjects, 885 had valid test results for both the Xpert HCV VL FS assay using capillary whole blood and Xpert HCV VL assay using EDTA plasma with an overall agreement of 97.4% (862/885). Of the 885, 433 were within the quantitation range of both assays. The result of the Deming regression analysis is shown below in Figure 8.

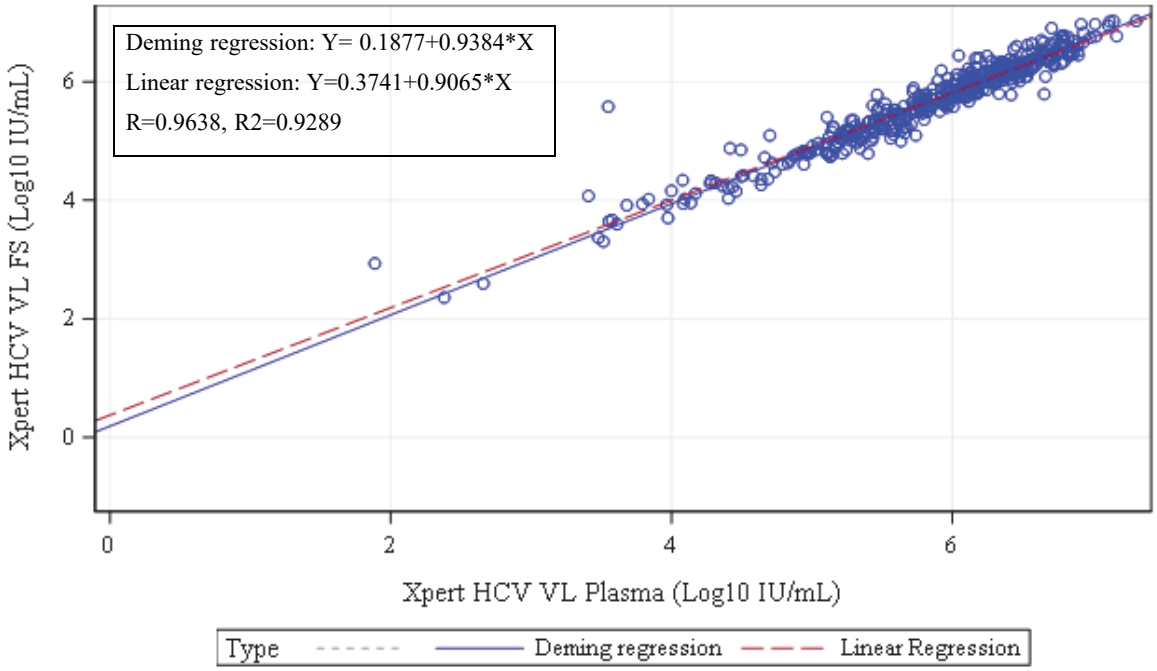

Figure 8. Xpert HCV VL FS Assay (Capillary WB) vs Xpert HCV VL (EDTA Plasma)

Of the specimens from 930 total subjects, 927 had valid test results for both Xpert HCV VL FS using venous whole blood and Xpert HCV VL using EDTA plasma with an overall agreement of 97.6% (905/927). Of the 927, 453 were within the quantitation range of both assays. The result of the Deming regression analysis is shown below in Figure 9.

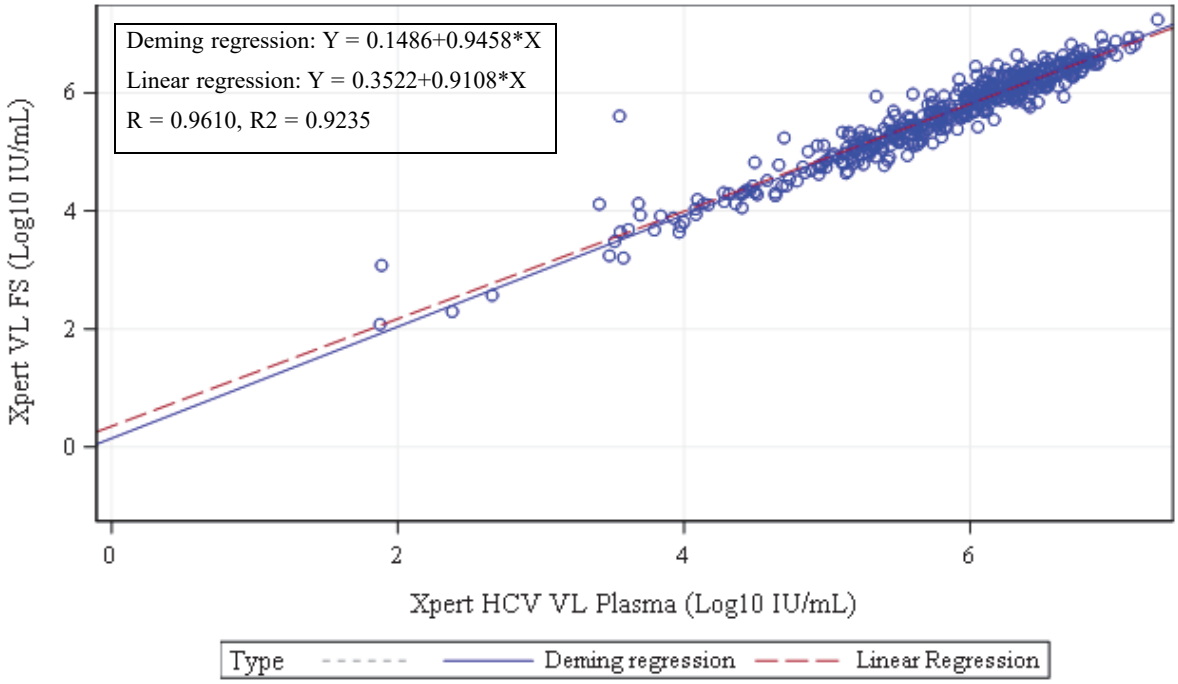

Figure 9. Xpert HCV VL FS Assay (Venous WB) vs Xpert HCV VL (EDTA Plasma)

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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
	CE Marking – European Conformity
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning

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