GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® HCV

REF **GXHCV-10**

Instructions for Use
For Use with GeneXpert Xpress System
CLIA Complexity: Waived

IVD

Cepheid®

In Vitro Diagnostic Medical Device

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See Section 26, Revision History for a description of changes.

Xpert® HCV

℞only

For *In Vitro* Diagnostic Use

CLIA Complexity: Waived

A Certificate of Waiver is required to perform this test in a CLIA-Waived setting. To obtain CLIA waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA. Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

1 Proprietary Name

Xpert® HCV

2 Common or Usual Name

Xpert HCV

3 Intended Use

The Xpert® HCV test, performed on the GeneXpert® Xpress System, is an automated *in vitro* reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of hepatitis C virus (HCV) RNA in human fingerstick K_2EDTA whole blood from adult individuals at risk and/or with signs and symptoms of HCV infection with or without antibody evidence of HCV infection. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection. Detection of HCV RNA does not discriminate between acute and chronic states of infection.

The Xpert HCV test is not intended for monitoring patients undergoing treatment or for use in screening blood, plasma, or tissue donors.

Warning: Performance characteristics have not been established for testing pregnant people or a pediatric population less than 22 years of age.

4 Summary and Explanation

Hepatitis C virus (HCV) is a small, positive-sense single-stranded RNA virus that belongs to the *Flaviviridae* family.[1] It is a major blood-borne human pathogen that causes chronic liver disease that can progress to cirrhosis, end-stage liver disease, and hepatocellular carcinoma.[2, 3] Approximately 58 million individuals are infected with HCV globally with approximately 300,000 deaths reported globally in 2019.[4] The HCV prevalence in the United States has been reported at approximately 1%[5] , with a range in HCV prevalence reported for Eastern Mediterranean (2.3%), Europe (1.5%), and Africa (1.0%).[2]

Transmission of HCV most commonly occurs from injection drug use through the sharing of injection equipment. Less commonly, HCV can also be transmitted from mother to child, and via sexual practices that lead to exposure to blood. Following initial infection, approximately 80% of people do not exhibit any symptoms. Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, pale feces, joint pain, and/or jaundice (yellowing of skin and the whites of the eyes).[6] Approximately 25% of infected individuals will spontaneously clear the virus within 6 months of infection without treatment. However, approximately 75% of HCV-infected individuals will develop chronic HCV infection.[3] Globally only 20% of HCV-infected individuals are aware of their HCV status, and

only 13% have been treated.[2] 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 possible in most patients with a highly effective, safe and tolerable combination of direct-acting antivirals (DAAs) taken for 8 - 12 weeks.[2, 7]

Guidelines recommend testing of individuals from high-risk populations and universal HCV screening for certain populations.[6, 8]

5 Principle of the Procedure

The Xpert HCV test, performed on the GeneXpert Xpress System, is an automated *in vitro* reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of hepatitis C virus (HCV) RNA in human fingerstick K_2EDTA whole blood from adult individuals at risk and/or with signs and symptoms for HCV infection with or without antibodies to HCV.

The Xpert HCV test includes reagents for the detection of HCV RNA in clinical specimens as well as a sample processing control (SPC) and internal control high (IC-H) 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 Sample Volume Adequacy (SVA) control ensures the sample was correctly added to the cartridge and verifies that the correct volume of sample has been added to the sample chamber.

With the GeneXpert Xpress System an operator can run the test by performing four steps: 1) mix the specimen, 2) transfer the liquid sample to the cartridge with a transfer pipette, 3) run the test on the instrument, and 4) read the results.

The GeneXpert Xpress System automates and integrates sample preparation, nucleic acid extraction, and amplification and detection of the target sequence in samples using real-time reverse transcription PCR. The GeneXpert Xpress System consists of an instrument, an integrated computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that contain the RT-PCR reagents and carry out the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the system, refer to the *GeneXpert Xpress System User's Guide*.

6 Materials Provided

The Xpert HCV test kit (GXHCV-10) contains sufficient reagents to process 10 specimens or quality control samples. Each kit contains the following:

Xpert HCV cartridges with integrated reaction tubes	**10 per kit**
• 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
• Binding Reagent	1.5 mL per cartridge
• Elution Reagent	1.5 mL per cartridge
Disposable 100 µL Transfer Pipettes	**20 per kit**
Instructions for Use	**1 per kit**
(For use with the GeneXpert Xpress System)	
Quick Reference Instructions	**1 per kit**
(For use with the GeneXpert Xpress System)	
CD	**1 per kit**
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert Xpress System	

Note Safety Data Sheets (SDS) are available at www.cepheid.com or 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 Xpert HCV test cartridges upright at 2-28°C until the expiration date provided on the label.
- If cartridge is stored refrigerated, bring the cartridge out and allow it to adjust to room temperature for at least 15 minutes, prior to use.
- Do not use expired cartridges.
- Do not open the cartridge until you are ready to perform the test.
- Do not use a cartridge that has leaked.
- Do not use cartridges that have been previously frozen.

8 Materials Required but Not Provided

- GeneXpert Xpress System (catalog number: GXIV-2-CLIA or GXIV-4-CLIA): GeneXpert Xpress IV instrument, GeneXpert Hub with proprietary GeneXpert Xpress Software Version 6.4a or higher, integrated computer, touchscreen monitor and barcode scanner, external CD drive, *Getting Started Guide*, and *GeneXpert Xpress System User's Guide*.
- Bleach or sodium hypochlorite
- Ethanol or denatured ethanol
- Absorbent pad
- High-flow lancet or equivalent (2 mm minimum depth, capable of yielding at least 250 µL of fingerstick whole blood)
- K_2EDTA-containing capillary collection tubes for small volumes (K_2EDTA microtainer BD part number: 365974)
- Alcohol wipe
- Gauze pad
- Bandage
- Warm pack

9 Materials Available but Not Provided

- NATtrol Hepatitis C Virus Negative Control, ZeptoMetrix Corporation catalog number NATHCVNEG-6C-IVD.
- NATtrol Hepatitis C Virus Positive Control, ZeptoMetrix Corporation catalog number NATHCV-6C-IVD.

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- For prescription 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[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. Consider use of face/eye protection.
- Wear clean lab coats and gloves.

- Reliable results are dependent on adequate specimen collection, transport, storage and processing, patient factors and/or state of infection. Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the amount of RNA in the specimen is below the limit of detection of the test. Careful compliance with the Instructions for Use and the *GeneXpert Xpress System Operator Manual* are necessary to avoid erroneous results.
- 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, transfer devices, and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.[11]
- Used cartridges may contain potentially infectious materials, as well as PCR amplicons. Do not open or alter any part of the used cartridge for disposal.
- Do not turn off or unplug the instrument while a test is in progress as this will stop the test.
- ONLY use the GeneXpert® Xpress System in recommended environmental operating conditions (59-86°F/15-30°C, 20-80% relative humidity).

10.2 Specimen

- For collection of fingerstick whole blood specimens, use only the K_2EDTA microtainer (BD part number: 365974). The microtainer should only be used to collect one specimen and should not be reused.
- For collection of fingerstick whole blood specimens please note that sample collection might be difficult in individuals with callused fingers. To adequately collect sufficient fingerstick whole blood from individuals with callused fingers, consider using deeper lancets.
- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under conditions other than those recommended has not been evaluated.
- Proper sample collection, storage, and transport are essential for correct results.

10.3 Assay/Reagent

- Do not use expired cartridges.
- Do not substitute Xpert HCV test reagents with other reagents.
- Do not open the Xpert HCV 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 cartridge lid may yield non-determinate results.
- Dropping or knocking over a cartridge after the sample is loaded may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the cartridge barcode label.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert HCV cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable transfer pipette is used to transfer one specimen. Do not reuse disposable transfer pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 1:10 dilution of freshly prepared household chlorine bleach. Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

11 Chemical Hazards[12]

- **Signal Word: DANGER**
- **GHS US Hazard Statements:**
  - Causes severe skin burns and eye damage.
  - Causes serious eye damage.
- **GHS US Precautionary Statements:**
  - **Prevention**
    - Do not breathe dust/fume/gas/mist/vapors/spray.
    - Wear protective gloves/protective clothing/eye protection/face protection.
  - **Response**
    - Immediately call a POISON CENTER or doctor.
    - IF ON SKIN: Take off immediately all contaminated clothing. Rinse skin with water (or shower).
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

12 Specimen Collection, Transport, and Storage

- Use a high-flow lancet to puncture the finger.
- Use the designated K_2EDTA microtainer for small volumes (BD part number: 365974) as per the manufacturer's instructions for use.
- Collect at least 250 µL and fill the microtainer to between the 250 µL and 500 µL marks on the reservoir.
- Proper specimen collection and storage and transport are critical to the performance of the Xpert HCV test. Specimen stability under shipping and storage conditions other than those listed below have not been evaluated with the Xpert HCV test.

12.1 Fingerstick Whole Blood Collection

1. Gather the necessary specimen collection supplies, including a high-flow safety lancet capable of yielding at least 250 µL of fingerstick blood (minimum 2 mm depth), the designated K_2EDTA-coated microtainer, an alcohol wipe, a gauze pad, a bandage, gloves, and a clean lab coat. Sample collection might be difficult in individuals with callused fingers. To adequately collect sufficient fingerstick whole blood from individuals with calluses on fingers, consider using deeper lancets.
2. Label the microtainer with a patient label without obscuring the volume markings on the tube.
3. It is critical to ensure the patient's hands are warm to the touch of others.
4. Warm the patient's hands with a warm pack, for at least 5 to 10 minutes. NOTE that failure to warm the hands is a major cause of insufficient blood flow and collection of insufficient volume of fingerstick blood.
5. Have the patient exercise their hands by clenching and unclenching it 10 times.
6. Clean the tip of the middle or ring finger with alcohol and allow it to air dry.
7. Remove the cap from the microtainer and place it on a convenient surface.
8. With the patient's palm facing the floor, place the lancet on the fingertip (slightly to one side), press the lancet firmly to the site.

Note If using a safety lancet, failure to press firmly before releasing the button has been shown to cause insufficient blood flow and collection of fingerstick blood.

9. Wipe the first drop of blood from the finger with a gauze pad. Keep the finger in a downward position.
10. Hold the microtainer tube at a 30-45° angle from the surface of the puncture site. Touch the collector end of the microtainer to the drop of blood.
11. Use gentle pressure and massage the finger and fingertip to initiate and maintain blood flow. Do not vigorously "milk" the finger as this may cause hemolysis.
12. It is important to gently tap the microtainer against the chair or counter in between drops to move blood to the bottom of the tube to avoid early clotting.

13. Fill the microtainer to between the 250 µL and 500 µL marks on the reservoir.
14. When sufficient blood has been collected, have the patient hold the gauze on their fingertip to stop the bleeding.
15. Replace the microtainer tube cap by twisting and pushing it downward until a snap is heard.
16. Immediately after collection, mix the sample by gently inverting the filled tube a minimum of 10 times (as recommended in the microtainer package insert).
17. Apply a bandage to the patient's finger.
18. Dispose of the lancet in an appropriate sharps' container.

Note A video (QR code) is available for sample collection guidance. This video must be viewed before collecting first patient sample. The video should be viewed again if sample collection issues are experienced.

12.2 Sample Transport and Storage

K_2EDTA fingerstick whole blood may be stored in the microtainer at 2-30°C for up to 4 hours prior to preparing the cartridge and testing.

13 Procedure

13.1 Starting the GeneXpert Xpress System

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Xpress System User's Guide*.

1. Put on a new pair of gloves.
2. Turn on the GeneXpert Xpress IV instrument (in two or four modules configuration).
3. Turn on the Hub computer. The Windows Lock screen appears.
4. Swipe up to continue. The Windows Password screen appears.
5. Touch **Password** to display the keyboard, and then type your Windows password.
6. Touch the arrow button at the right of the password entry area. The GeneXpert Xpress Software starts, and a login screen appears.
7. If enabled, you may log in by scanning a barcode on your institutional ID, using the barcode scanner (located behind the right side of the touchscreen). Then proceed to Step 10. Otherwise, follow the steps below to login manually.
8. Enter your User Name and Password (the virtual keyboard appears once you touch the entry fields).
9. Touch the **X** in the upper right of the virtual keyboard. The keyboard disappears and the **LOGIN** button appears at the bottom of the screen. Touch the **LOGIN** button to continue.
10. The Database Maintenance Reminder screen and the Archive Tests Reminder dialog boxes may appear, depending on your system configuration. For more information, see the *GeneXpert Xpress System User's Guide*.

14 Starting the Test using GeneXpert Xpress System

14.1 Starting a Test

Note Before you start the test, make sure that the system is running GeneXpert Xpress software version 6.4a or higher and that the Xpert HCV Assay Definition File is imported into the software.

The following instructions showing how to prepare the sample and the cartridge are shown on-screen in a video and are also described in the *Quick Reference Instructions* (QRI).

1. Put on a new pair of gloves if performing a new test.
2. Touch the **NEW TEST** button on the Home screen (see Figure 1).

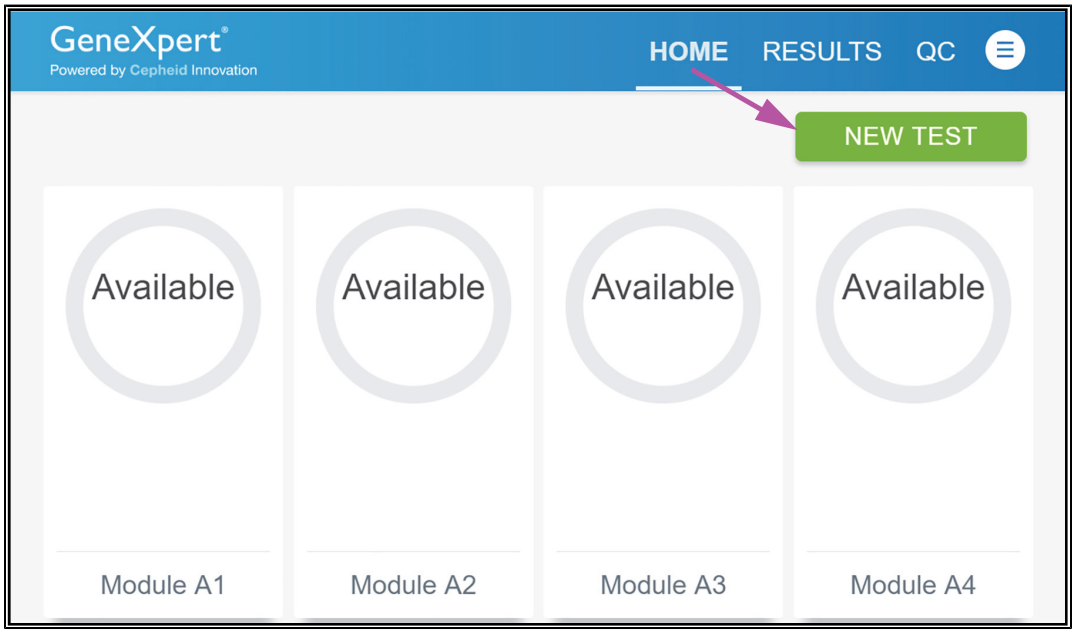

Figure 1. The Home Screen

If Patient Information is configured by an administrator, then the Patient Information screen appears (see Figure 2). If Patient Information is not configured, the Sample ID screen appears.

3. Skip to Section 14.2 if the Sample ID screen appears.

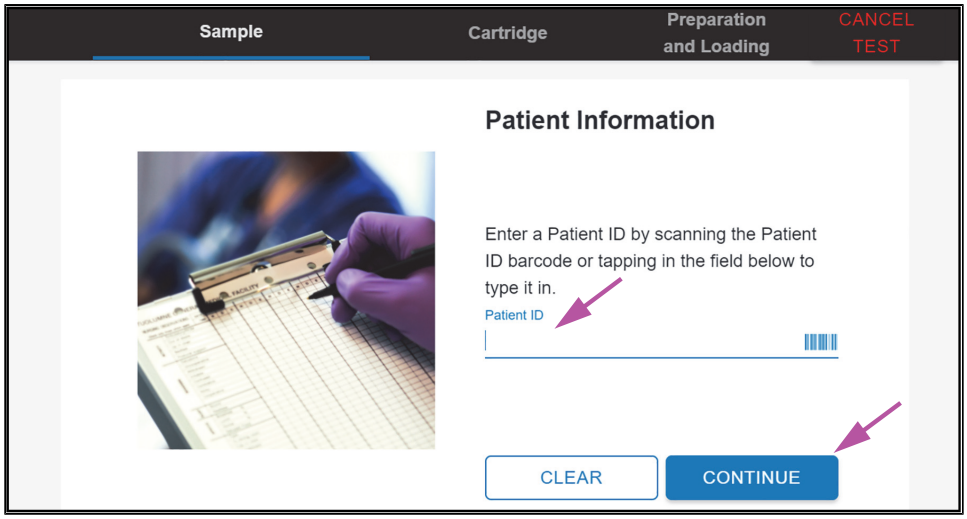

Figure 2. Patient Information Screen

4. Scan Patient ID barcode or manually enter the Patient ID.
5. Touch **CONTINUE**. The Confirm Patient Information screen appears.
6. Verify the Patient ID and touch **CONFIRM**. The Sample ID screen appears.

14.2 Preparing the Specimen

1. Scan Sample ID barcode or manually enter the Sample ID.
2. Touch **CONTINUE**. The Confirm Sample ID screen appears (see Figure 3).

Figure 3. Confirm Sample ID Screen

3. Verify the Sample ID and touch **CONFIRM**. The Scan Cartridge Barcode screen appears (see Figure 4).

Important **In the following steps, cartridges should be kept upright when handling or scanning. Do not rotate or tip the cartridge, because damage to the contents or injury to personnel may occur.**

Note

If the barcode on the Xpert HCV cartridge does not scan or scanning the barcode results in an error message stating the cartridge is expired, then use a new cartridge.

If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating the assay definition file is not loaded on the system. If this screen appears, contact Cepheid Technical Support.

Figure 4. Scan Cartridge Barcode Screen

4. Obtain a Xpert HCV cartridge from the Xpert HCV cartridge kit box and scan the cartridge barcode. After scanning, the Confirm Test Information screen appears.
5. Review and confirm test information by touching **CONFIRM**. Depending on your configuration, the Enter Credentials to Continue screen may appear. If enabled, you may log in by scanning your institutional ID. Otherwise, manually enter your User Name and Password and touch **LOGIN** to continue.
6. The Cartridge Preparation screen appears.
7. A video clip shows the cartridge preparation steps and is available for guidance on loading the sample into the test cartridge. This video must be viewed before loading the first sample to a cartridge. The video should be viewed again if sample loading issues are experienced.
8. Once complete, the video starts from the beginning automatically. Touch the **CONTINUE** button to exit video. Prepare the cartridge according to the directions below (See Section 14.3), which are also shown in the video.

14.3 Cartridge Preparation and Loading the Cartridge

Important **Start the test immediately after adding the sample to the cartridge.**

1. Wear protective disposable gloves.
2. Remove a transfer pipette from the Xpert HCV cartridge kit box.
3. The Xpert HCV cartridge obtained in Section 14.2 should be allowed to adjust to room temperature prior to use.
4. Inspect the test cartridge for damage. If damaged, do not use it.
5. Label the cartridge with sample identification. Do not place the Sample ID label on the cartridge lid or on the cartridge barcode label.

Loading Fingerstick Whole Blood Sample into Cartridge

1. Gather the necessary supplies, including the microtainer sample, a packaged transfer pipette from the Xpert HCV kit, an Xpert HCV cartridge labeled with a patient label, and an absorbent pad.
2. Open the pipette wrapper, leaving the pipette inside.
3. Open the cartridge lid.
4. Check that the microtainer is fully closed and mix the sample by gently inverting the tube 10 times. If the sample has been sitting in the microtainer for more than 15 minutes, gently invert it 20 times.
5. Carefully uncap the microtainer and put the cap onto the absorbent pad, blood side up.
6. Remove the pipette from the wrapper.
7. Squeeze the top bulb of the pipette completely until the two sides of the bulb touch and place the pipette tip in the microtainer.
8. Keeping the pipette tip below the surface of the liquid and close to the bottom of the microtainer, release the top bulb of the pipette slowly and fully until it is completely filled with sample before removing it from the microtainer. It is OK if liquid goes into the overflow reservoir of the pipette.
9. **Check that the pipette does not contain air bubbles (see Figure 5). If air bubbles are present, dispense blood back into the microtainer, keeping the pipette tip above the surface of the liquid while dispensing, and repeat this step with a new pipette. Note that air bubbles in the pipette may trigger the instrument to abort the run due to insufficient volume added to the cartridge.**

Note Loading less than 100 µL blood into the cartridge may trigger an insufficient volume error (ERROR 2097).

Important **Make sure NOT to aspirate air into the pipette after the pipette has been lifted from the blood surface in the microtainer, as this can lead to insufficient blood volume (see Figure 5). Do NOT pour the specimen into the chamber! Discard the pipette appropriately after use.**

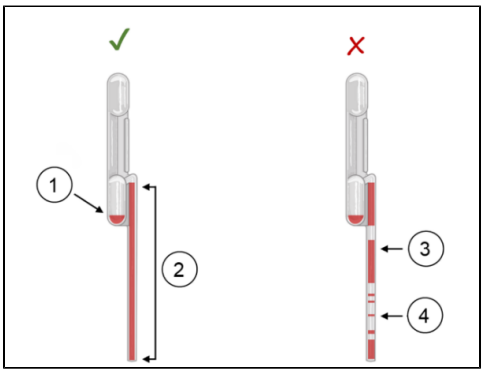

Figure 5. Xpert HCV Test 100 µL Transfer Pipette (Correct and Incorrect Use)

Number	Description
1	Overflow Reservoir with excess sample
2	100 µL blood (sample)
3	Rapid pipetting may result in air bubbles
4	Air pocket

10. Place the pipette tip deep into the sample chamber of the cartridge (Figure 6).

Note There is a thin plastic film that covers the inner ring of the 13 ports of the test cartridge. This film should not be removed.

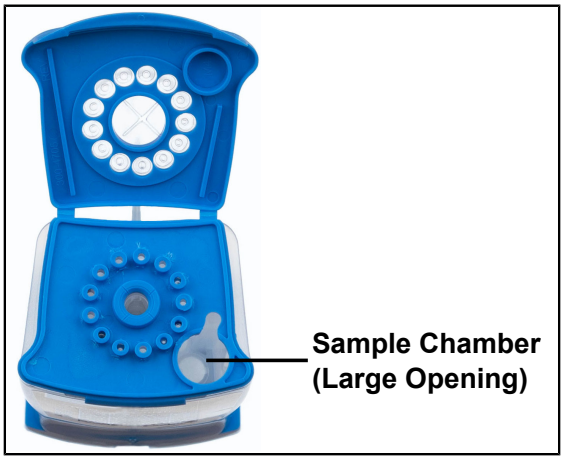

Figure 6. Xpert HCV Cartridge (Top View)

11. Squeeze the top bulb of the pipette completely until the two sides of the bulb touch to deliver the sample to the bottom of the sample chamber in the cartridge. Some liquid may remain in the overflow reservoir of the pipette.
12. Continue to squeeze the top bulb firmly and do not release until the pipette is removed from the cartridge.
13. Dispose the used pipette in a biohazard container.
14. Close the cartridge lid. Ensure the lid snaps firmly into place.
15. Replace the microtainer cap by twisting and pushing it downward until a snap is heard.
16. Change gloves and prepare to load the cartridge onto the instrument (See Section 14.4).

Note

A video (QR code) is available for guidance on loading the sample into the test cartridge. This video must be viewed loading the first sample to a cartridge. The video should be viewed again if sample loading issues are experienced. The same video is also available in Step 7 of Section 14.2.

14.4 Loading the Cartridge

1. Touch the **CONTINUE** button on the Cartridge Preparation screen. The **Load Cartridge into Module** screen appears.
2. Open the module door with the flashing green light.
3. Load the cartridge with the barcode facing the operator onto the cartridge bay platform. Do not try to insert the cartridge past the cartridge bay platform.
4. Close the module door. The green light stops flashing, and the test starts. When the cartridge is loaded, the Test Loading screen appears, followed by the Test Running screen showing that the test is running.

   When the test is done, the green light goes out and the module door automatically unlocks. The screen text changes to Test Completed (see Figure 7 and Figure 8).

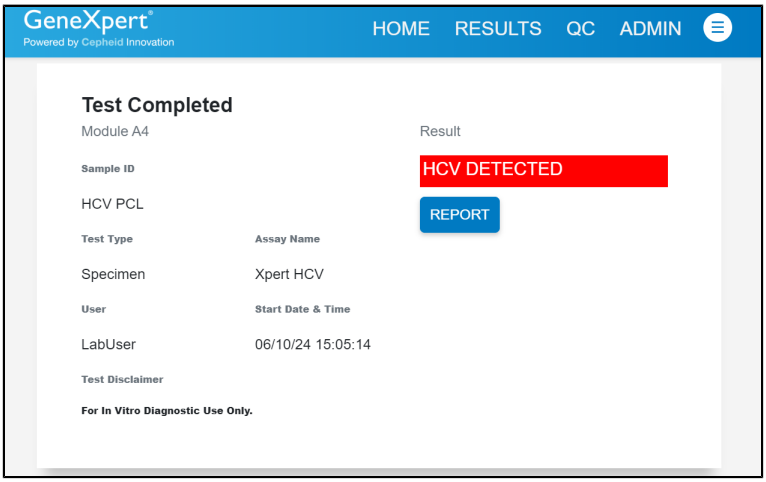

Figure 7. Test Result Screen: HCV DETECTED (Example)

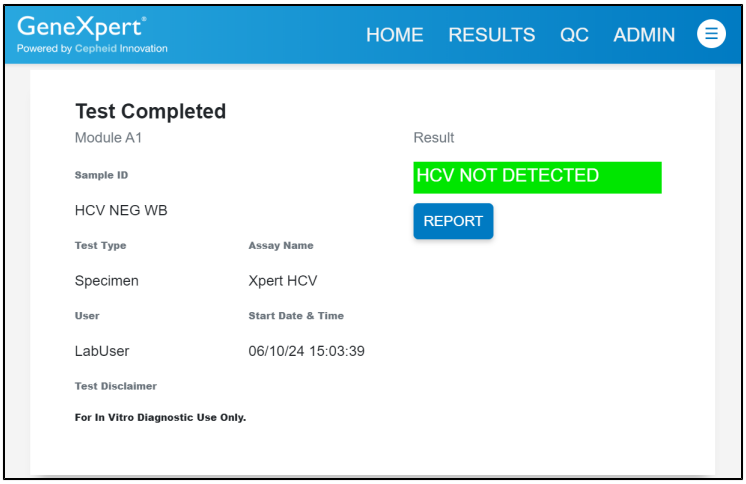

Figure 8. Test Result Screen: HCV NOT DETECTED (Example)

The **Test Completed** screen provides the results for the test just completed.

5. Open the module door, remove the used cartridge, and properly dispose of the cartridge according to your institution's hazardous waste disposal policies.
6. Touch **REPORT** to view the result of the test that has just complete. Touch **HOME** to go back to the home screen. This completes the procedure for running a test. To log out, touch the **User Menu** Icon, then select **Logout**.

14.5 Starting a New Test while a Test is Running

You can start a new test while another test is in progress.

1. Put on a new pair of gloves if performing a new test.
2. Touch the **HOME** button on the Test Running screen.
3. For a new user login, touch the **User Menu** icon to log in.
4. Repeat the steps in Section 14.1, Starting the Test through Section 14.4, Loading the Cartridge.
5. After a second test has started, touch the **HOME** button. The status of both tests appears. The Home screen displays the module(s) in use with a circular graphic indicator around each test, and Patient Identification below the module graphic (see Figure 9).

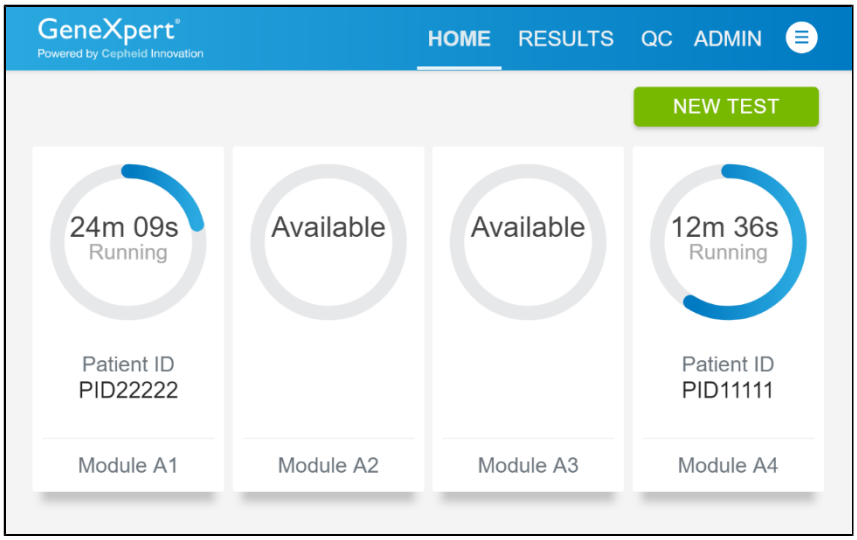

Figure 9. Home Screen Showing Two Tests Running

After a test has completed, the module icon text changes to Complete (see Figure 10).

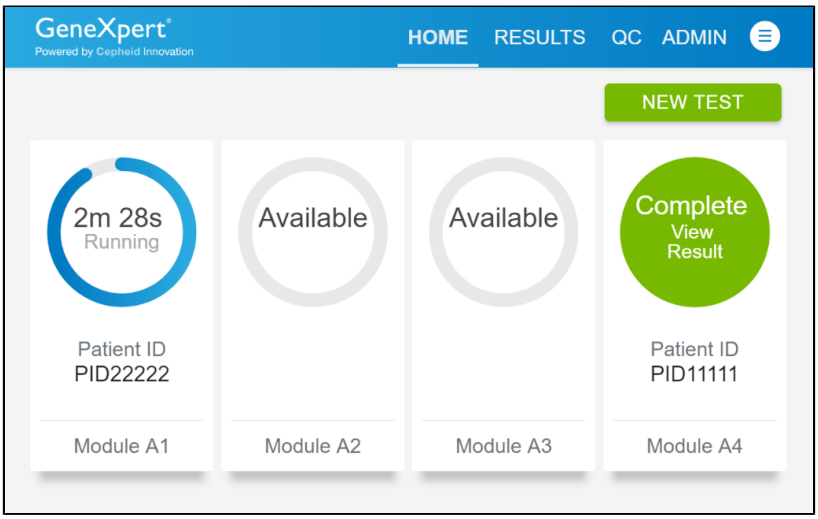

Figure 10. Home Screen with One of Two Tests Completed

14.6 Viewing Test Results

1. Touch the **RESULTS** button located on the panel at the top of the screen (see Figure 9 and 10). The Results screen appears (see Figure 11).

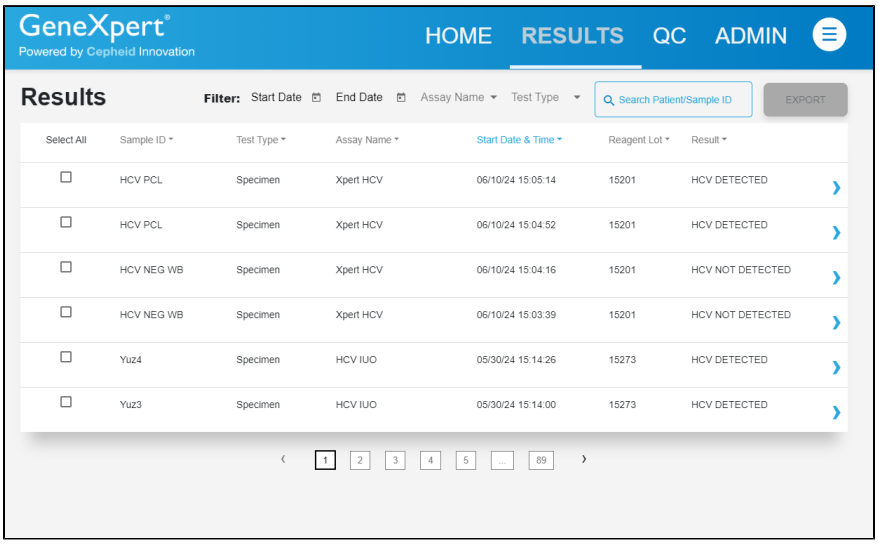

Figure 11. Result Screen (Example)

Test results are, by default, in order by date and time that the test was run. Navigate through the test result pages by touching the numbered buttons or arrows at the bottom of the screen.

2. Touch the desired result to open the Test Result screen (see Figures 7 and 8).

14.7 Running External Controls

It is recommended that external controls be tested at the frequency listed below:

- Each time a new lot of Xpert HCV kits is received.
- Each time a new shipment of Xpert HCV kits is received even if it is the same lot previously received.
- Each time a new operator is performing the test (i.e., operator who has not performed the test recently).
- When problems (storage, operator, instrument, or other) are suspected or identified.
- If otherwise required by your institution's standard Quality Control (QC) procedures.

The negative and positive external control is in a ready to use format, proceed to Step 1.

1. Put on a new pair of gloves.
2. Have a new Xpert HCV test cartridge, a transfer pipette provided in the Xpert HCV test kit, and a quality control tube ready.
3. On the home screen or the Test Running screen, touch **QC** (see Figure 12).

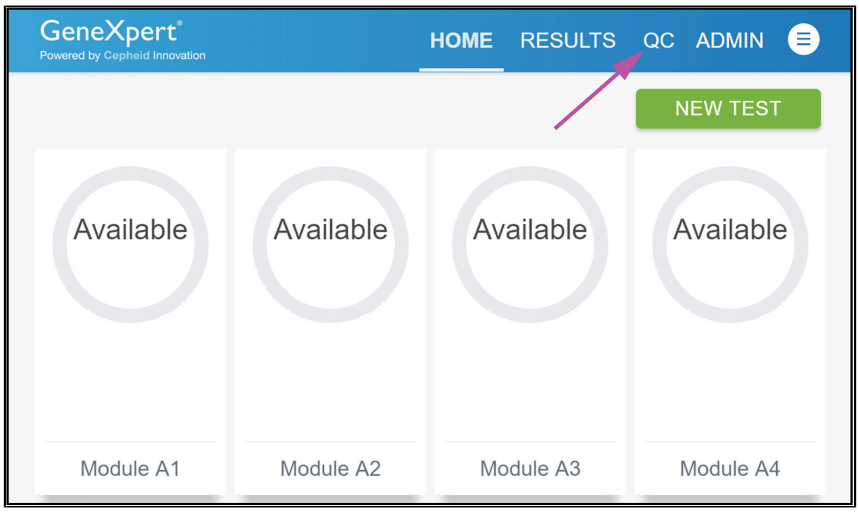

Figure 12. Home Screen

4. The Quality Control screen appears. Touch Run QC Positive Test or Run QC Negative Test (see Figure 13) based on the test being performed.

Figure 13. Quality Control Screen

5. The Sample ID screen appears.
6. Manually enter “Negative Control” for the Negative Control or “Positive Control” for the Positive Control or scan the sample ID barcode.
7. Touch **CONTINUE**. The Confirm Sample ID screen appears.
8. Verify the Sample ID and touch **CONFIRM**. The Scan Cartridge Barcode screen appears (see Figure 14).

Figure 14. Scan Cartridge Barcode Screen

Important **In the following steps, keep the cartridges upright when handling or scanning. Do not rotate or tip the cartridge, because damage to the content or injury to personnel may occur.**

9. Select the appropriate cartridge and scan the cartridge barcode. After scanning, the Select Test Information screen appears.
10. Confirm that the test information is correct, then touch **CONFIRM**.
11. Bring the external control tube to room temperature prior to use.
12. Check that the external control tube cap is closed.
13. Mix the external control material by gently inverting the external control tube 20 times. Open the lid of the external control tube.
14. Open the cartridge lid by lifting the front of the cartridge lid.
15. Remove the pipette from the wrapper.
16. Squeeze the top bulb of the pipette completely until the two sides of the bulb touch and place the pipette tip in the external control tube.
17. Keeping the pipette tip below the surface of the liquid, release the top bulb of the pipette slowly and fully until it is completely filled with external control material before removing it from the external control tube. It is OK if liquid goes into the overflow reservoir of the pipette.
18. **Check that the pipette does not contain air bubbles (Figure 15). If air bubbles are present, dispense the external control material back into the tube, keeping the pipette tip above the surface of the liquid while dispensing, and repeat this step with a new pipette.**

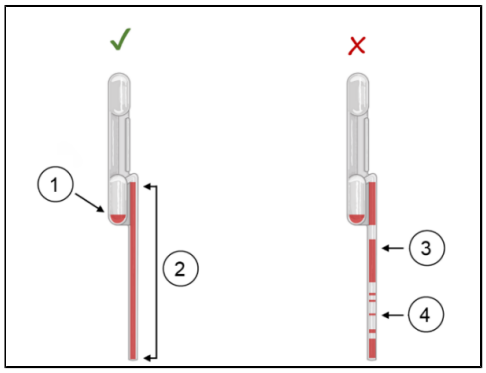

Figure 15. Xpert HCV Test 100 µL Transfer Pipette (Correct and Incorrect Use)

Number	Description
1	Overflow Reservoir with excess sample
2	100 µL blood (sample)
3	Rapid pipetting may result in air bubbles
4	Air pocket

19. Place the pipette tip deep into the sample chamber of the cartridge (Figure 16).

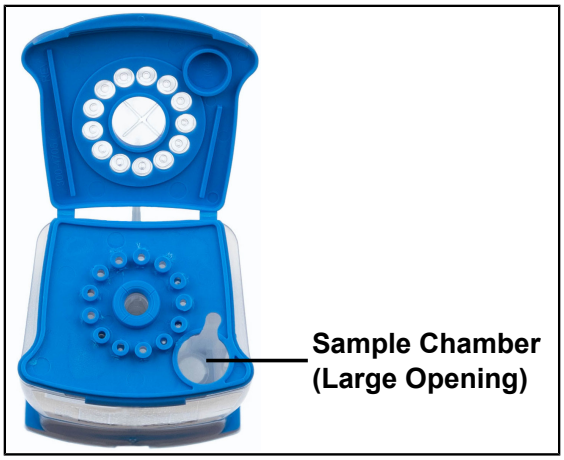

Figure 16. Xpert HCV Cartridge (Top View)

20. Squeeze the top bulb of the pipette completely until the two sides of the bulb touch to deliver the external control material to the bottom of the sample chamber. Some liquid may remain in the overflow reservoir of the pipette.
21. Continue to squeeze the top bulb firmly and do not release until the pipette is removed from the cartridge.
22. Dispose the used pipette in a biohazard container.
23. Close the cartridge lid.
24. Close the external control tube.
25. Change gloves and prepare to load the cartridge onto the instrument.
26. Touch the **CONTINUE** button on the Cartridge Preparation screen. The Load Cartridge into Module screen appears.
27. Open the module door with the flashing green light.
28. Load the cartridge with the barcode facing the operator onto the cartridge bay platform. Do not try to insert the cartridge past the cartridge bay platform.
29. Close the module door. The green light stops flashing, and the test starts. When the cartridge is loaded, the Test Loading screen appears, followed by the Test Running screen showing that the test is running.

Note

When the test is done, the green light goes out and the module door automatically unlocks. The screen text changes to Test Completed.

The Test Completed screen provides the results for the test just completed.

30. Open the module door, remove the used cartridge, and properly dispose of the cartridge according to your institution's hazardous waste disposal policies.
31. Touch **REPORT** to view the result of the test that has just completed. Touch **HOME** to go back to the home screen. This completes the procedure for running an external quality control. To log out, touch the **User Menu** Icon, then select **Logout**.

15 Quality Control

15.1 Internal Controls

Each test includes a Sample Volume Adequacy (SVA) control, sample processing control (SPC), an internal control high (IC-H), and a 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, **NO RESULT-REPEAT TEST** will be displayed. The SVA error can be caused by cartridge-related error associated with insufficient sample volume. The system will prevent the test from being processed.
- **Sample Processing Control (SPC) and Internal Control High (IC-H) –** The SPC and IC-H are two RNA controls unrelated to HCV that are included in each cartridge and go through the whole test process. They ensure that the sample was correctly processed and detect specimen-associated inhibition of the RT-PCR. The SPC and IC-H should PASS in a negative sample and be N/A in a positive sample. The SPC and IC-H pass if they meet the validated acceptance criteria.
- **Probe Check Control (PCC)** – Before the start of the PCR, the GeneXpert Xpress 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.

15.2 External Controls

External controls described in Section 9 are available but not provided and must be used in accordance with local, state, and federal accrediting organizations' requirements, as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert Xpress System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the Test Result screen for GeneXpert Xpress System (Figure 7 and Figure 8). Possible results are shown in Table 1.

Table 1. Xpert HCV Test Results and Interpretation for GeneXpert Xpress System

Result	Interpretation
HCV DETECTED	HCV RNA is detected.
HCV NOT DETECTED	HCV RNA is not detected.
NO RESULT - REPEAT TEST	If the result is **NO RESULT - REPEAT TEST**, then retest with a new cartridge using a new transfer pipette.*
INSTRUMENT ERROR	Result is an instrument error. Touch **CLEAR ERROR** and follow the on-screen instructions. When the Home screen appears, repeat the test using a new cartridge and a new transfer pipette.

*** Do not perform more than one retest of a sample.**

The Xpert HCV test includes an Early Assay Termination (EAT) function which will provide earlier time to results if the signal from the target nucleic acid reaches a predetermined threshold before all PCR cycles have been completed.

17 Retests

17.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test according to the instructions in Section 17.2, Retest Procedure.

- An **INSTRUMENT ERROR** result could be due to (but is not limited to), exceeding the maximum pressure limits.
- A **NO RESULT - REPEAT TEST** indicates that insufficient data were collected. For example, the Probe Check Control failed or an internal control failed in an HCV negative sample or insufficient sample volume was added to the cartridge or a power failure occurred on the instrument.

If an External Control fails to perform as expected, repeat the external control test and/or contact Cepheid Technical Support for assistance.

17.2 Retest Procedure

1. To retest a **NO RESULT - REPEAT TEST** or **INSTRUMENT ERROR** result (non-determinate result), use a new cartridge (do not re-use the original cartridge).
2. Repeat the test once using a new cartridge and a new transfer pipette (supplied).
3. Do not perform more than one retest of the original specimen.
4. See Section 13, Procedure, including Section 13.1, Starting the GeneXpert Xpress System, and Section 14, Starting the Test using GeneXpert Xpress System.
5. If the same error occurs, contact Cepheid Technical Support for troubleshooting assistance. Contact information is in Section 24, Technical Assistance.

18 Limitations

- The Xpert HCV test has been validated for use with the GeneXpert Xpress System using only the procedures provided in this Instructions for Use. Modification to these procedures may alter the performance of the test.
- The Xpert HCV test has been validated with fingerstick whole blood collected with the K_2EDTA-containing capillary collection tubes for small volumes (K_2EDTA microtainer BD part number: 365974). Use of the Xpert HCV test with specimen types other than fingerstick whole blood may result in inaccurate test results.
- Erroneous test results might occur from improper specimen collection, technical error, sample mix-up, or because the HCV RNA level in the specimen is not detected by the test. Careful compliance with the instructions in this Instructions for Use and the BD microtainer instruction documents are necessary to avoid erroneous results.
- A negative test result does not exclude the possibility of infection because test results may be affected by improper specimen collection, technical error, specimen mix-up, or the HCV RNA level in the specimen that may be below the sensitivity of the assay.
- The Xpert HCV test provides qualitative results.
- The Xpert HCV test performance has been evaluated only in adult patients.
- The Xpert HCV test is not cleared for monitoring patients undergoing treatment or for use in screening blood, plasma, or tissue donors.
- The Xpert HCV test performance characteristics have not been established in populations of immunocompromised or immunosuppressed patients or, other populations where test performance may be affected.
- As with many diagnostic tests, results from the Xpert HCV test should be interpreted by qualified licensed healthcare professionals in conjunction with the individual's clinical presentation, history, and other laboratory results.
- Mutations or other changes within the regions of the HCV genome covered by the primers and/or probes in the Xpert HCV test may result in failure to detect the target organisms.
- Drug interference studies were performed *in vitro* and may not assess the potential interferences that might be seen after the drugs are metabolized *in vivo*.
- There was limited evaluation of the Xpert HCV performance in non-viral hepatitis patients (e.g., NASH, Alcoholic Liver Disease and Autoimmune Hepatitis); therefore, their potential cross-reactivity is unknown.
- There was limited evaluation of the Xpert HCV performance in patients with HBV.

19 Performance Characteristics

19.1 Clinical Performance

A prospective clinical study was conducted to evaluate the performance of the Xpert HCV test at 15 CLIA-Waived sites from geographically diverse locations in the US with 32 untrained users participating in the study. Participants included individuals ≥ 22 years of age who were at risk and/or with signs and symptoms for HCV infection. A total of 1012 fingerstick whole blood specimens were obtained from 1012 eligible participants for testing with the Xpert HCV test. In addition, 982 serum specimens were tested on an FDA-approved nucleic acid amplification test (NAAT) and an FDA-approved HCV antibody test. Patient management continued at the site per the standard practice, independent of investigational test.

The Xpert HCV test performance was compared to a patient infected status (PIS) algorithm based on results from the FDA-approved HCV NAAT and the FDA-approved HCV antibody test and the results are shown in Section 19.2, Tables 2, 3, 4, and 5.

19.2 Results

The study population was comprised of 1012 patients 22 years of age and above. Table 2 below presents the total number of participants by risk factor:

Table 2. Summary of Participants by Risk Factor

Risk Factor[a]	n/N (%)
Intravenous drug use	422/1012 (41.7%)
Persons who are HIV-positive	148/1012 (14.6%)
Blood transfusion or organ transplant received prior to 1992	12/1012 (1.2%)
Blood clotting factor for hemophilia received prior to 1987	1/1012 (0.1%)
Born between 1945 and 1965	406/1012 (40.1%)
Born to an HCV-infected mother	5/1012 (0.5%)
Chronic hemodialysis patients	3/1012 (0.3%)
Persons who engage in high-risk sexual behavior	111/1012 (11.0%)
Persons with known exposure to HCV, such as Healthcare workers after needle sticks involving HCV-positive blood and Recipients of blood or organs from a donor who tested HCV-positive	53/1012 (5.2%)

[a] Participants may have reported multiple risk factors

Available demographic data collected from study participants are presented in Table 3.

Table 3. Demographic Data Summary of Participants

Demographic Characteristics		Overall (N=1012)
Age	≥ 22 years old ≤ 60	646 (63.8%)
	> 60	366 (36.2%)
Gender	Male	544 (53.8%)
	Female	468 (46.2%)
Race[a]	Black/African American	369 (36.5%)
	White	521 (51.5%)

Demographic Characteristics		Overall (N=1012)
	Asian	10 (1.0%)
	Other[b]	93 (9.2%)
	Unknown/Prefer not to answer	18 (1.8%)
	Missing	1 (0.1%)
Ethnicity	Hispanic Latino	130 (12.8%)
	Not Hispanic Latino	862 (85.2%)
	Unknown/Prefer not to answer	16 (1.6%)
	Not Available/Missing	4 (0.4%)
History of HCV infection	Yes	293 (29.0%)
	No	717 (70.8%)
	Missing	2 (0.2%)
Recent HCV antibody test	Yes	544 (53.8%)
	Not Available	158 (15.6%)
	Never had a HCV antibody test	308 (30.4%)
	Missing	2 (0.2%)
Results of recent HCV antibody test	Reactive	164 (16.2%)
	Not Reactive	376 (37.2%)
	Invalid	3 (0.3%)
	No Result[c]	469 (46.3%)
Recent HCV NAAT test	Yes	204 (20.2%)
	Not Available	196 (19.4%)
	Never had a HCV NAAT test	611 (60.4%)
	Missing	1 (0.1%)
Result of recent HCV NAAT Test	Positive	34 (3.4%)
	Negative	170 (16.8%)
	No Result[d]	808 (79.8%)
HCV Genotype Test	Yes	46 (4.5%)
	Not Available	246 (24.3%)
	Never had a HCV genotyping test	719 (71.0%)
	Missing	1 (0.1%)
Genotype Result	1a	30 (3.0%)
	1b	6 (0.6%)
	1c	2 (0.2%)
	2b	2 (0.2%)
	3a	5 (0.5%)
	No Result[e]	967 (95.6%)

Demographic Characteristics		Overall (N=1012)
Treatment History	Not currently treated	175 (17.3%)
	Never been treated	836 (82.6%)
	Missing	1 (0.1%)
Symptomatic	Yes	373 (36.9%)
	No	639 (63.1%)
At risk	Yes	934 (92.3%)
	No	78 (7.7%)
Symptomatic and at risk	Yes	295 (29.2%)
	No	717 (70.8%)
History of injection drug use	Yes	437 (43.2%)
	No	575 (56.8%)
History of Non-HCV liver disease	Yes	68 (6.7%)
	No	943 (93.2%)
	Missing	1 (0.1%)
HIV Status	Positive	154 (15.2%)
	Negative	799 (79.0%)
	Unknown (Never tested)	59 (5.8%)
HBV Status	Positive	16 (1.6%)
	Negative	684 (67.6%)
	Unknown (Never tested)	311 (30.7%)
	Missing	1 (0.1%)

[a] If more than one race is reported for a participant, they are only captured in one category.

[b] Other race group includes "American-Indian or Alaskan Native", "More than one race", "Native Hawaiian or Pacific Islander" and "Other"

[c] Combined category of "Not Available", "Never had a HCV Antibody Test" and "Missing"

[d] A combined category of "Not Available", "Never had a HCV NAAT Test" and "Missing"

[e] A combined category of "Not Available", "Never had a HCV Genotyping Test" and "Missing"

Of the 1,012 eligible samples, 30 samples were excluded due to the following reasons: 1) protocol deviations (n=15); 2) unresolved ND results for Xpert HCV test (n=11); and 3) non-evaluable comparator test results (n=4). A total of 982 samples tested were included in the Xpert HCV test performance calculations. A total of 982 fingerstick whole blood specimens were eligible and tested for inclusion in the Xpert HCV clinical study. Table 4 presents the Xpert HCV test results by patient infected status (PIS).

Table 4. Xpert HCV Test Results according to Patient Infected Status

HCV Ab	HCV RNA NAAT	Patient Infected Status	Xpert HCV	N
Reactive	Reactive	Active chronic infection	HCV DETECTED	111
			HCV NOT DETECTED	6
	Non-reactive	Past/resolved infection	HCV DETECTED	1
			HCV NOT DETECTED	223
Non-reactive	Reactive	Active acute infection	HCV DETECTED	3
			HCV NOT DETECTED	2
	Non-reactive	Not infected	HCV DETECTED	1
			HCV NOT DETECTED	635

Performance of the Xpert HCV test is presented in Table 5. The Xpert HCV test demonstrated positive percent agreement (PPA) and negative percent agreement (NPA) of 93.4% and 99.8%, respectively when compared to the PIS (Table 5).

Table 5. Agreement between Xpert HCV Test Results with the Patient Infected Status

		Patient Infected Status		
		HCV Positive[a]	**HCV Negative[b]**	**Total**
Xpert HCV Test	**HCV detected**	114	2[c]	116
	HCV not detected	8[c]	858	866
Total		122	860	982
PPA[c]		93.4% (95% CI: 87.6 – 96.6)		
NPA[c]		99.8% (95% CI: 99.2 – 96.9)		

[a] Active chronic or acute infection.

[b] Past/resolved infection or not infected.

[c] Two (2) specimens (1 false positive and 1 false negative) with suspicion of specimen handling and testing errors were retested along with 110 additional serum specimens on the HCV RNA NAAT comparator test (15 positive and 95 negative). Retesting confirmed the specimen handling and testing error at the reference laboratory.

19.3 Non-Determinate Rate

Of the 1012 Xpert HCV runs performed in the clinical study, 61 resulted in non-determinate ("INSTRUMENT ERROR" or "NO RESULT - REPEAT TEST") results on first attempt. A total of 58 out of the 61 specimens were retested and nine (9) specimens remained non-determinate. Including the 3 specimens with initial non-determinate result without retesting, 12 specimens had final non-determinate results. The initial non-determinate rate was 6.0% (61/1012) and the final non-determinant rate was 1.2% (12/1012).

19.4 Testing Non-Viral Hepatitis Samples

Fingerstick samples from individuals with other liver diseases (where active HCV infection was not indicated as the underlying cause) were collected during the clinical study. Samples were tested with an FDA-approved anti-HCV test and an FDA-approved HCV molecular test to confirm the samples were HCV negative. Seventy-eight (78) samples from individuals with non-viral hepatitis were included in the study.

Table 6 below shows subject demographics and liver disease status for the 78 evaluable subjects.

Table 6. Non-Viral Hepatitis Disease Group Clinical Performance by Disease

Non-viral Liver Disease[a]	N of Xpert Test by Disease Occurrence	N of Xpert Test Compared to PIS[b]	TP	FN	TN	FP	PPA with 95% CI (%)	NPA with 95% CI (%)
Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)	28	24	1	0	23	0	100.0% (95% CI: 20.7 – 100.0)	100.0% (95% CI: 85.7 – 100.0)
NASH	2	1	0	0	1	0	NA	100.0% (95% CI: 20.7 – 100.0)
Primary biliary cirrhosis	15	13	2	0	11	0	100.0% (95% CI: 34.2 – 100.0)	100.0% (95% CI: 74.1 – 100.0)
Chronic HBV	3	2	0	0	2	0	NA	100.0% (95% CI: 34.2 – 100.0)
Alcoholic liver disease	11	10	0	0	10	0	NA	100.0% (95% CI: 72.2 – 100.0)
Autoimmune hepatitis	3	3	0	0	3	0	NA	100.0% (95% CI: 43.9 – 100.0)
Other	16	15	1	0	14	0	100.0% (95% CI: 20.7 – 100.0)	100.0% (95% CI: 78.5 – 100.0)
Total	**78**	**68**	**4**	**0**	**64**	**0**	**100.0% (95% CI: 51.0 – 100.0)**	**100.0% (95% CI: 94.3 – 100.0)**

[a] Patients may have multiple non-viral liver diseases
[b] With both valid Xpert test result and valid PIS results

20 Analytical Performance

20.1 Analytical Sensitivity (Limit of Detection)

Limit of Detection (LoD): The LoD of the Xpert HCV test was determined by testing dilutions of the 6th WHO International Standard (NIBSC Code: 18/184) for hepatitis C Virus RNA for genotypes 1a and clinical isolates for genotypes 1b, 2b, 3a, 4, 5 and 6 in HCV negative human fingerstick whole blood. Probit analysis was used to determine the LoD. The LoD for each genotype is shown in Table 7 below:

Table 7. LoD of Xpert HCV for genotypes 1a, 1b, 2b, 3a, 4, 5, and 6, in Fingerstick Whole Blood

Genotype	Fingerstick whole blood LoD (IU/mL)	95% Confidence Interval (IU/mL)
1a	35.0	(22.3 – 54.9)
1b	41.5	(29.8 – 57.9)
2b	89.2	(47.7 – 166.8)
3a	58.2	(39.0 – 86.7)
4	32.2	(21.1 – 49.1)
5	136.4	(83.2 – 223.4)
6	83.8	(57.2 – 122.8)

20.2 Analytical Specificity (Cross-reactivity)

The following viruses and microorganisms were evaluated for potential cross-reactivity in the Xpert HCV test. Organisms were diluted to a concentration of 100,000 CFU/mL, copies/mL, or $TCID_{50}$/mL as applicable. Each organism in the panel was tested in the presence (3-7x Limit of Detection (LoD)) and absence of HCV RNA. Positive and negative HCV controls tested in replicates of 6 were included in the study.

None of the organisms tested in this study (Table 8) showed cross reactivity with HCV detection using the Xpert HCV test in HCV-positive and HCV-negative samples.

Table 8. Organisms Tested for Analytical Specificity

Virus	Bacteria	Fungus
Human Immunodeficiency virus 1 (HIV-1)	*Staphylococcus aureus*	*Candida albicans*
Human Immunodeficiency virus 2 (HIV-2)	*Streptococcus epidermidis*	
Human T-cell Lymphotropic virus Type 1 (HTLV-I)		
Human T-cell Lymphotropic virus Type 2 (HTLV-II)		
Dengue Virus		
West Nile Virus		
Zika Virus		
Banzi Virus		
Ilheus Virus		
Yellow Fever Virus		
Cytomegalovirus		
Epstein-Barr Virus (EBV)		
Hepatitis A Virus (HAV)		
Hepatitis B virus (HBV)		
Herpes simplex Virus 1 (HSV-1)		
Herpes simplex Virus 2 (HSV-2)		
Human Herpesvirus 6 (HHV-6)		
Human Herpesvirus 8 (HHV-8)		
Varicella Zoster Virus (VZV)		
BK Human Polyoma Birus		
Human papilloma Virus 16 (HPV-16)		
Human papilloma Virus 18 (HPV-18)		
St. Louis Encephalitis Virus		
Vaccinia Virus		

20.3 Potentially Interfering Substances

The susceptibility of the Xpert HCV test to interference by elevated levels of potentially interfering substances was evaluated. Endogenous substances, exogenous substances, and autoimmune disease specimens were tested in the presence (300 IU/mL) and absence of HCV RNA. Each endogenous and exogenous substance was tested in replicates of six, and the autoimmune disease specimens in replicates of three. Two controls without any potentially interfering substance consisting of HCV negative K_2EDTA venous WB and HCV negative K_2EDTA venous WB spiked with HCV to 300 IU/mL was also included and tested in replicates of six. This study was conducted using one kit lot.

No interference in the performance of the Xpert HCV test was observed in the presence of the substances evaluated at the concentrations listed in Table 9-Table 11 below.

Table 9. Endogenous Interferents Tested

Endogenous Substance	Concentration for Testing
Albumin	9 g/dL
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Human DNA	0.4 mg/dL
Triglycerides	3,000 mg/dL

Table 10. Exogenous Interferent Pools Tested

Drug Pool	Generic Name	Concentration for Testing ($3xC_{max}$)	Tested as:
Pool 1	Zidovudine	6.00 µg/mL	Drug
	Abacavir sulfate	11.67 µg/mL	Drug
	Saquinavir	0.59 µg/mL	Drug
	Ritonavir	44.40 µg/mL	Drug
	Interferon 2b	819.00 IU/mL	Drug
	Ombitasvir	0.20 µg/mL	API
	Paritaprevir	0.79 µg/mL	API
	Dasabuvir	2.00 µg/mL	API
Pool 2	Fosamprenavir	17.16 µg/mL	Drug
	Peginterferon alfa-2a	0.08 µg/mL	Drug
	Peginterferon alfa-2b	0.00032 µg/mL	Drug
	Ribavirin	10.70 µg/mL	Drug
	Ledipsavir	0.97 µg/mL	API
	Sofosbuvir	1.85 µg/mL	API
	Daclatasvir	4.60 µg/mL	API
	Simeprevir	5.81 µg/mL	API
Pool 3	Tenofovir disoproxil fumarate	1.17 µg/mL	Drug
	Lamivudine	6.00 µg/mL	Drug
	Indinavir sulfate	35.57 µg/mL	Drug

Drug Pool	Generic Name	Concentration for Testing ($3xC_{max}$)	Tested as:
	Ganciclovir	31.20 µg/mL	Drug
	Acyclovir	68.70 µg/mL	Drug
	Valganciclovir HCl	34.44 µg/mL	Drug
Pool 4	Stavudine	2.05 µg/mL	Drug
	Efavirenz	15.72 µg/mL	Drug
	Lopinavir	40.50 µg/mL	Drug
	Enfuvirtide	18.27 µg/mL	Drug
	Ciprofloxacin	4.77 µg/mL	API
	Clarithromycin	5.10 µg/mL	Drug
	Maraviroc	1.00 µg/mL	Drug
Pool 5	Nevirapine	7.20 µg/mL	Drug
	Nelfinavir	14100.00 µg/mL	API
	Azithromycin	2.10 µg/mL	Drug
	Valacyclovir	24.06 µg/mL	Drug

API: Active Pharmaceutical Ingredient

Table 11. Autoimmune Disease Samples Tested

Condition	Number of Specimens
SLE / ANA positive	7
SLE / ANA negative	2
SLE / ANA unknown	3
RA / RF positive	8

SLE: Systemic Lupus Erythematosus, ANA: Anti-Nuclear Antibodies, RA: Rheumatoid Arthritis, RF: Rheumatoid Factor

20.4 Carry-over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent specimen and amplicon carry-over contamination from very high titer positive samples into successively run negative samples when processed in the same GeneXpert module.

A high titer HCV positive specimen (1,000,000 IU/mL) was tested, immediately followed by testing an HCV negative specimen in the same GeneXpert instrument module. The procedure was repeated twenty (20) times in two different modules. Each replicate of the HCV negative specimen, processed immediately following a replicate of the high titer HCV positive specimen, was correctly reported “HCV Not Detected”. No evidence of specimen or amplicon carry-over contamination was observed.

20.5 Precision

The lot-to-lot variability of the Xpert HCV test was established through a single site, blinded, and randomized precision study using three lots of the Xpert HCV cartridges. The study tested three clinical HCV strains from genotype (GT) 1a, GT2b, and GT3a and external controls (one positive and one negative). Three analyte levels were tested for each genotype: negative, ~1.5x LoD, and ~3.0x LoD. The study was conducted with two trained operators, over 5 independent days of testing (not necessarily consecutive), with two runs per day (1 run per operator per day), and two replicates per sample per

run, using one GeneXpert Xpress System (four module system) with the Hub configuration (GeneXpert Xpress software version 6.4a). The total number of replicates per sample tested was 60. Table 12 presents the percent correct for each panel level, using Fisher's Exact test.

Table 12. Summary of Precision Percent Agreement Results for the Xpert HCV Test

Panel Member	% Agreement[a]	95% CI
Negative	100% (60/60)	94.0% – 100%
GT1a 1.5 x LoD	100% (60/60)	94.0% – 100%
GT1a 3.0 x LoD	100% (60/60)	94.0% – 100%
GT2b 1.5 x LoD	95.0% (57/60)	86.3% – 98.3%
GT2b 3.0 x LoD	100% (60/60)	94.0% – 100%
GT3a 1.5 x LoD	100% (59/59)[b]	93.9% – 100%
GT3a 3.0 x LoD	100% (60/60)	94.0% – 100%

a Number of Replicates with Expected Results / Number of Valid Replicates. All replicates provided valid results.

b One (1) sample yielded non-determinate results on initial test and on retesting.

The precision of the Xpert HCV test was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) are provided for each covariate: within-run (repeatability), between-runs, between-days, between-lots, and total variance (within-laboratory precision) for each panel member are presented in Table 13.

Table 13. Summary of Ct Variance Components Observed in the Precision Study

Target and Level	Analyte of Ct Values	N[a]	Mean Ct	Repeatability (Within run)		Between Runs		Between Days		Between Lots		Total Variance (Within-Laboratory)	
				SD	CV (%)[b]	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	60	35.09	0.43	1.23	0.00	0.00	0.22	0.64	0.35	0.99	0.60	1.70
GT1a 1.5 x LoD	HCV	60	38.43	0.91	2.36	0.25	0.64	0.00	0.00	0.35	0.92	1.00	2.61
GT1a 3.0 x LoD	HCV	60	37.89	0.71	1.87	0.22	0.58	0.35	0.93	0.29	0.76	0.87	2.30
GT2b 1.5 x LoD	HCV	57	39.77	1.20	3.02	0.00	0.00	0.35	0.88	0.52	1.30	1.35	3.40
GT2b 3.0 x LoD	HCV	60	38.44	0.70	1.82	0.47	1.23	0.00	0.00	0.16	0.41	0.86	2.23
GT3a 1.5 x LoD	HCV	59	38.43	0.87	2.27	0.00	0.00	0.14	0.36	0.43	1.13	0.99	2.56
GT3a 3.0 x LoD	HCV	60	37.27	0.53	1.41	0.00	0.00	0.22	0.59	0.00	0.00	0.57	1.53

a N is the total number of non-zero Ct values

b CV (%) = SD/Mean Ct * 100

The Xpert HCV test demonstrated acceptable precision per panel member between runs, between days and between lots when testing was performed in a laboratory environment.

20.6 Reproducibility

The reproducibility of the Xpert HCV test was established through a multicenter (3 CLIA-Waived sites), blinded and randomized study, using a single reagent lot of the Xpert HCV cartridges. The study tested three clinical HCV strains from genotype (GT) 1a, GT2b, and GT3a and external controls (one positive and one negative). Three analyte levels were tested for each genotype: negative, ~1.5x LoD, and ~3.0x LoD. The study was conducted with three untrained operators per site, during 5 independent days of testing, with one run per day (1 run per operator per day), and two replicates per sample per run, using one GeneXpert Xpress System (four module system) with the Hub configuration (GeneXpert Xpress software version 6.4a) at 3 sites. The total number of replicates per sample tested was 90. Table 14 presents the percent correct for each panel level, using Fisher's Exact test.

Table 14. Summary of Reproducibility Percent Agreement Results for the Xpert HCV Test

Target and Level	Site 1				Site 2				Site 3				Agreement by Target
	Op 1	Op 2	Op 3	Site Total	Op 1	Op 2	Op 3	Site Total	Op 1	Op 2	Op 3	Site Total	(%; 95% CI)
Negative	100% (10/10)	100% (10/10)	90% (9/10)	96.7% (29/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	98.9% (94.0%-99.8%) 89/90
GT1a 1.5 x LoD	100% (10/10)	90% (9/10)	100% (10/10)	96.7% (29/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	98.9% (94.0%-99.8%) 89/90
GT1a 3.0 x LoD	100% (10/10)	100% (10/10)	90% (9/10)	96.7% (29/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	98.9% (94.0%-99.8%) 89/90
GT2b 1.5 x LoD	100% (10/10)	90% (9/10)	100% (10/10)	96.7% (29/30)	100% (10/10)	90% (9/10)	100% (10/10)	96.7% (29/30)	100% (10/10)	90% (9/10)	100% (10/10)	96.7% (29/30)	96.7% (90.7%-98.9%) 87/90
GT2b 3.0 x LoD	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (95.9%-100%) 90/90
GT3a 1.5 x LoD	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (95.9%-100%) 90/90
GT3a 3.0 x LoD	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (95.9%-100%) 90/90

The reproducibility of the Xpert HCV test was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) within-run (repeatability), between-days, between-operators, between-sites, and total reproducibility for each panel member are presented in Table 15.

Table 15. Summary of Ct Variance Components Observed in the Reproducibility Study

Target and Level	Analyte of Ct Values	N[a]	Mean Ct	Repeatability (Within Run)		Between Days		Between Operators		Between Sites		Reproducibility	
				SD	CV (%)[b]	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	90	35.05	0.44	1.25	0.00	0.00	0.27	0.76	0.00	0.00	0.51	1.46
GT1a 1.5x LoD	HCV	89	38.42	0.90	2.33	0.00	0.00	0.36	0.95	0.00	0.00	0.97	2.52
GT1a 3.0x LoD	HCV	89	37.63	0.68	1.82	0.00	0.00	0.28	0.74	0.00	0.00	0.74	1.96

Target and Level	Analyte of Ct Values	N[a]	Mean Ct	Repeatability (Within Run)		Between Days		Between Operators		Between Sites		Reproducibility	
				SD	CV (%)[b]	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
GT2b 1.5x LoD	HCV	87	39.53	0.91	2.31	0.45	1.14	0.00	0.00	0.43	1.08	1.10	2.79
GT2b 3.0x LoD	HCV	90	38.43	0.73	1.90	0.08	0.20	0.06	0.17	0.22	0.57	0.77	2.00
GT3a 1.5x LoD	HCV	90	38.18	0.82	2.15	0.20	0.52	0.25	0.67	0.00	0.00	0.88	2.31
GT3a 3.0x LoD	HCV	90	37.14	0.74	1.99	0.00	0.00	0.22	0.58	0.00	0.00	0.77	2.07

[a] N is the total number of non-zero Ct values

[b] CV (%) = SD/Mean Ct * 100

The Xpert HCV test demonstrated acceptable reproducibility across sites, operators, days and panel members when testing was performed in a CLIA-Waived environment.

21 CLIA Waiver Studies

The performance of the Xpert HCV test was evaluated when used by untrained operators who had no CLIA Moderate / High Complexity laboratory experience and were representative of operators from a CLIA-Waived environment in a prospective all-comers study. The clinical study was conducted at 15 CLIA-Waived sites from geographically diverse locations in the US with 32 untrained operators participating. The performance of Xpert HCV test was determined relative to the patient infection status and the results are shown in Section 19.2, Table 4.

Near Cutoff Study

The near cutoff study was incorporated into the reproducibility study. The study was conducted to evaluate the performance of Xpert HCV with weakly reactive samples when tested by untrained operators. This blinded study was performed at three external sites representative of a CLIA-Waived environment and utilized a multi-factor nested design consisting of contrived panel members spanning the relevant limit of detection (LoD) for three HCV genotypes. The panel testing was conducted over a minimum of five days at each site. The performance of Xpert HCV with samples near the assay cutoff was acceptable when tested by untrained operators and are shown in Section 20.6, Table 14.

Flex Studies

Using risk analysis as a guide, flex studies were conducted on Xpert HCV for use with the GeneXpert Xpress System. The testing evaluated numerous sources of potential human errors that could affect the accuracy of results, including those related to sample handling, cartridge handling, and the operation of the GeneXpert Xpress System. The studies demonstrated that the Xpert HCV test and the GeneXpert Xpress System are robust to the usage variation that may be encountered.

22 References

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24 Technical Assistance

Before Contacting Us

Collect the following information before contacting Cepheid Technical Support:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

Contact Information

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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en_US/support/contact-us.

25 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Expiration date
	Temperature limitation
	For prescription use only

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26 Revision History

Description of Changes: 303-3318, Rev. A

Purpose: Initial release of Instructions for Use for the Xpert HCV test.