CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW240017

B. Parent Document Number

DEN240016

C. CLIA Waiver Type:

CLIA Waiver by Application

D. Applicant

Cepheid

E. Proprietary and Established Names

Xpert HCV test
GeneXpert Xpress System

F. Measurand (analyte)

Hepatitis C Virus (HCV) RNA in human fingerstick whole blood

G. Sample Type(s)

Human K_2EDTA fingerstick whool blood

H. Type of Test

Automated Qualitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

I. Test System Description

1. Overview

The Xpert HCV test, is an automated qualitative *in vitro* reverse transcription polymerase chain reaction (RT-PCR) test. The Xpert HCV test is performed on the GeneXpert Xpress System. With this system an operator can run the test by performing four simple steps: 1) mix the specimen, 2) transfer the blood sample to the cartridge with a transfer pipette, 3) run the test on the instrument, and 4) read the results.

The GeneXpert Xpress System (Hub configuration) consists of a GeneXpert IV instrument that executes the sample preparation, nucleic acid amplification and real-time fluorescent signal detection for the test, and a GeneXpert Hub with preloaded GeneXpert Xpress software for running the tests and viewing the results. The GeneXpert Hub accessory integrates the computer, touchscreen monitor and barcode scanner. Each of the GeneXpert modules in the GeneXpert IV instrument can perform separate sample preparation and testing. The module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE (Intelligent Cooling/Heating Optical Reaction) thermocycler for performing real-time PCR and RT-PCR and detection.

The Xpert HCV test requires the use of a single-use disposable GeneXpert cartridge that contains all necessary reagents for the detection of HCV RNA. Because the cartridges are self-contained, the risk of cross-contamination between samples is minimized. 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.

The Xpert HCV test is designed for use with human K_2EDTA fingerstick whole blood. The BD Microtainer for capillary whole blood collection was validated for use with the Xpert HCV test. After collecting human K_2EDTA fingerstick whole blood in the BD Microtainer, a 100µl aliquot of the specimen is transferred to the sample chamber of the Xpert HCV cartridge using the transfer pipette supplied in the Xpert HCV kit.

The sample results are interpreted by the GeneXpert Xpress System from measured fluorescent signals and embedded calculation algorithms and are shown in the View Results window. It also reports if the test has encountered an instrument error or produces no result and needs to be repeated.

This device was De Novo granted in DEN240016. Additionally, the GeneXpert Xpress System was previously cleared (in K231381, K173398, K180218, and K171552) and CLIA waived (in CW230013, CW190007, CW190006, CW180002, CW170014, and CW170005). The current submission is to obtain a CLIA waiver for the DEN240016 test device, the Xpert HCV Test.

2. Test System Components

The Xpert HCV test Kit contains sufficient cartridges to process 10 samples from patients 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 GenXpert Xpress System

Material not included with the kit but available separately:

- GeneXpert Xpress System (catalog number: GXIV-2-CLIA or GXIV-4-CLIA), including GeneXpert Hub with integrated computer running proprietary GeneXpert Xpress software version 6.4a or higher, touchscreen monitor and barcode scanner, external CD drive, Getting Started Guide, and GeneXpert Xpress System User's Guide.
- High-flow lancet or equivalent (2 mm minimum depth, capable of yielding at least 250 µL of capillary whole blood).
- EDTA-containing capillary collection tubes for small volumes (K_2EDTA microtainer BD part number: 365974).
- External Positive and Negative controls
- Alcohol wipes
- Gauze pad
- Bandage
- Warm pack

3. Workflow

The Xpert HCV test, performed on the GeneXpert Xpress System, is an automated qualitative *in vitro* reverse transcription polymerase chain reaction (RT-PCR) test for the detection of hepatitis C Virus (HCV) RNA in human K2-EDTA fingerstick whole blood. The Xpert HCV test single-use disposable cartridge includes reagents for the detection of HCV RNA in clinical specimens as well as a sample processing control (SPC), internal control high (IC-H), the Probe Check Control (PCC), and the Sample Volume Adequacy (SVA). After collecting human K2-EDTA fingerstick whole blood in the BD Microtainer, a 100µl aliquot of the specimen is transferred to the sample chamber of the Xpert HCV cartridge using the transfer pipette supplied in the Xpert HCV kit. The cartridge is loaded onto the GeneXpert Xpress System platform. The instrument then performs automated sample processing followed by amplification, detection, and reporting of results. The Xpert HCV test uses three channels to detect target organism and two internal controls (SPC and IC-H). The results are interpreted automatically by the GeneXpert Xpress System from measured fluorescent signals and embedded calculation algorithms.

4. Result Interpretation

The GeneXpert Xpress System displays results in the View Results window. It also reports if the test has encountered an instrument error or produces no result and needs to be repeated. Test results are obtained in approximately 56 minutes. 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 and 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 the 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.

J. Demonstrating "Simple"

- The Xpert HCV is a self-contained test: PCR reagents and processes are hosted in single use, self-contained GeneXpert cartridges.
- The test uses direct unprocessed specimens: human capillary EDTA whole blood.
- Specimen manipulation is non-technique dependent. Capillary whole blood specimens are collected in the K_2EDTA microtainer, mixed by inversion and transferred into test cartridges. Pre-analytic sample handling requirements for the Xpert HCV test are simple specimen patient ID checks and sample transfer with a fixed volume transfer pipette. No precise measuring is required.
- Reagent manipulation is non-technique-dependent: PCR reagents are preloaded and automatically processed within the GeneXpert cartridges. The test cartridges are keyed and can be inserted into the analyzer only in one direction.
- The test does not require any operator intervention during the analysis step. When the Xpert HCV test is complete, results are displayed by the Xpress software at the touch of a button.
- Technical or specialized training is not required for troubleshooting or error code interpretation. If an error code is shown, simple on-screen instructions are provided to the user. In addition, the GeneXpert Xpress user's guide provides information regarding error handling and provides the phone number for contacting technical support. The Xpert HCV Package Insert includes the explanations and actions that should be taken by the user. Retests are indicated for "INSTRUMENT ERROR" or "NO RESULT – REPEAT TEST" results.
- Required electronic or mechanical maintenance tasks are simple. GeneXpert Xpress instrument users perform basic cleaning procedures. Cepheid recommends that the system be checked for proper calibration on an annual basis. If an error code is shown, the user's guide provides information regarding error handling and provides the phone number for contacting Cepheid for

technical support. A System Control Check for temperature ensures that the GeneXpert Xpress instrument is operating within validated heating and cooling specifications.

- Test results do not require operator calibration, interpretation, or calculation. The analytic test and the post-analytic results are displayed automatically, neither of which require any additional interpolation or calculation.
- The Gene Xpert Xpress System screen is designed for ease of use and features a color display that facilitates easy-to-read messages. The results are reported on a screen as "HCV DETECTED", "HCV NOT DETECTED". Non-reportable results are displayed as "NO RESULT-REPEAT TEST" or "INSTRUMENT ERROR" and there is no interpretation required by the end-user. Error messages are unambiguous and include easy-to-interpret solutions.
- The Quick Reference Instructions (QRI) and the GeneXpert Xpress *Getting Started Guide* are written at a 7th grade comprehension level. In addition, two videos (QR codes) are available for sample collection guidance and for loading the cartridge.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

   Risk analysis was performed by Cepheid using the Failure Modes and Effects Analysis (FMEA) Method according to ISO 14971 "*Medical devices - Application of risk management to medical devices*". The risk analysis was included in the submission. Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions in the labeling. All risks of harm to the patient or operator were mitigated to an acceptable level and were supported by flex studies and/or operator instructions.

2. Fail-Safe and Failure Alert Mechanisms

   The Xpert HCV test performed on the GeneXpert Xpress System was designed with several fail-safe and failure alert mechanisms to prevent erroneous results as described in Table 2.

   Table 2. Summary of Fail-Safe and Failure Alert Mechanisms of the GeneXpert Xpress System.

Design Feature	Description
Operator Lockout	• Module door will not latch if the reaction tube of the cartridge is incorrectly positioned. Incorrect position can also be detected by force increase and the test will not run. The test will also not run without a cartridge inserted. • Module door closes before test starts to block external light; there is also a signal check for light leak. • Module door will not close if an attempt to run a test on a GeneXpert Xpress module is made without a blinking green light. Only a GeneXpert module with a blinking green light can be used to start a new test. • Only one test can be started at a time.

Design Feature	Description
Instrument Self-Test	• The GeneXpert Xpress System has an internal function of on-going internal performance monitoring and if the data indicate that maintenance is required, the operator will be instructed to contact Cepheid Technical Support, in which case the company will send a support technician to the operator. • Self-check performed by the software before the test starts includes thermal checks for temperature out of range, checks of the heating rate and cooling rate, check of the force sensor for cartridge loading, optics check, syringe drive and valve checks.
GeneXpert Xpress Instrument	• The GeneXpert Xpress instrument has an ambient temperature sensor that monitors the internal operating temperature and is designed to prevent the test from proceeding when the ambient temperature of the module is above 55°C. • The system should be checked for proper calibration on an annual basis using the Xpert Check kit. If an error code is shown, the operator is instructed to contact Cepheid for technical support.
Consumable Design	The test barcode is read once the cartridge is scanned: • The instrument will not start if the test cartridge has previously been used on the same instrument. • The instrument will not start if the Assay Definition File of the test cartridge scanned is not loaded. • The instrument will not start if the test cartridge is expired.

Fixed Volume Transfer Pipette

The risk of using an incorrect sample volume is minimized by the fixed volume pipette that is included with the kit.

External Controls

The commercially prepared external positive and negative controls are available, but are packaged and provided separately. The Package Insert and Quick Reference Instructions provide recommendations on when the external controls should be tested.

Internal Controls

The Xpert HCV test is a PCR-based Nucleic Acid Amplification Test. Each test requires the use of a single-use disposable GeneXpert cartridge that contains all necessary reagents for the detection of HCV RNA. Because the cartridges are self-contained, the risk of cross-contamination between samples is minimized. The following internal controls are also included in the cartridge:

- 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 a 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) 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 reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity and dye stability. The PCC passes if it meets the validated acceptance criteria.

Self-Checks

The GeneXpert Xpress has an internal function for on-going internal performance monitoring. If the data indicate that maintenance is required, the user will be instructed to contact Cepheid Technical Support, in which case Cepheid will send a support technician to the testing site.

The functionality of the fail-safe mechanisms built into the software of the Xpert HCV test on the GeneXpert Xpress System were tested as described below in Table 3. For any error messages that may appear on-screen, the user is instructed to follow the on-screen instructions.

Table 3. Fail-Safe Mechanisms for the Xpert HCV Test with the GeneXpert Xpress Instrument.

Item	Operator Action/Condition	Expected Results
1	Ambient temperature of the module is above 55°C.	The GeneXpert Xpress software will detect a **hardware error**, which will be indicated by a red error light on the module. The testing module icon displayed on the "Home" screen will be marked "Error H/W Failed" with a full orange circle and cannot be selected to run a test, indicating a test cannot be performed on that module. Testing does not proceed. The Getting Started Guide informs the user to contact Cepheid Technical Support.
2	Test was stopped by touching the "STOP TEST" icon before results were obtained.	**Confirm Message:** "Test is currently running. Would you like to stop the test?" Test results are reported as "NO RESULT – REPEAT TEST" following the stopping of the test.
3	Test was stopped by touching the "STOP TEST" icon before results were obtained and then the operator attempted to resume the test with the same cartridge.	**First Message:** "Test is currently running. Would you like to stop the test?" Test results are reported as "NO RESULT – REPEAT TEST" following the stopping of the test. **Second Message** following resuming of the run using the same cartridge: "Cartridge serial number [#########] for assay with product code [###] reagent lot [#####] has already been used. Cartridges can only be used once. Select a new cartridge."

Item	Operator Action/Condition	Expected Results
		Testing does not proceed.
4	Operator turned off (unplugged) the instrument from an electrical outlet before the test was completed followed by restoration of power to theinstrument.	**Error Message** following restoration of power to the instrument: "Code 2123: Module AX lost communication while test was running, attempting recovery [DATE XX/XX/XX, TIME XX:XX:XX].Test results are reported as "INSTRUMENT ERROR." The package insert and QRI contain a warning to not turn off or unplug the instrument while a test is in progress.
5	Operator turned off (unplugged) the instrument before the test was completed and tried to resume the test once the instrument was back on.	**First Error Message:** "Code 2123: Module AX lost communication while test was running, attempting recovery [DATE XX/XX/XX, TIME XX:XX:XX]." **Second Error Message:** "Cartridge serial number [#########] for assay with product code [###] reagent lot [#####] has already been used. Cartridges can only be used once. Select a new cartridge." Testing does not proceed.
6	Operator attempts to run cartridges beyond the expiration date.	**Error Message:** "Cartridge expired on [YYYY/MM/DD]. Please use valid Cartridge." The user is unable to proceed past the screen and the test cannot be started. The operator can touch the OK button and scan an unexpired cartridge, or the operator can touch the "CANCEL TEST" button to return to the HOME screen. There is a note in the package insert to not use expired cartridges.
7	Cartridge was dropped after adding sample.	Test proceeds but "NO RESULT – REPEAT TEST" is obtained (possible **error code**: Sample Volume Adequacy (SVA) error (Error 2097) or Cartridge Integrity Test (CIT) error (Error 2037)). The package insert and QRI contain a warning to the user that the cartridge should not be used in testing if

Item	Operator Action/Condition	Expected Results
		it is dropped after the addition of sample.
8	Cartridge was shaken after adding sample	Test proceeds but "NO RESULT – REPEAT TEST" is obtained (possible **error code**: Sample Volume Adequacy (SVA) error (Error 2097)). The package insert and QRI contain a warning to not shake or tilt the cartridge after the addition of sample.
9	Cartridge reaction tube is missing.	Test proceeds but "NO RESULT – REPEAT TEST" is obtained (possible **error code**: Cartridge Integrity Test (CIT) error (Error 2037)). The package insert and QRI contain a warning to the operator that the cartridge should not be used if the cartridge reaction tube is missing.
10	Cartridge reaction tube is damaged.	Test proceeds but "NO RESULT – REPEAT TEST" is obtained (possible **error code**: Cartridge Integrity Test (CIT) error (Error 2037)). The package insert and QRI contain a warning to the operator that the cartridge should not be used if the cartridge reaction tube is damaged.
11	Start a test using a cartridge that has already been used on the same instrument as the spent cartridge.	**Error Message:** "Cartridge serial number [#########] for assay with product code [###] reagent lot [#####] has already been used. Cartridges can only be used once. Select a new cartridge." Testing does not proceed. The package insert and QRI contain a warning to the operator to not reuse processed cartridges
12	Start a test using a cartridge that has already been used on a different instrument as the spent cartridge.	Test proceeds but "NO RESULT – REPEAT TEST" is obtained (possible **error code**: Sample Volume Adequacy (SVA) error (Error 2097)).

3. Flex Studies

Flex studies were performed to evaluate the robustness of the GeneXpert Xpress System and Xpert HCV test reagents as well as variations in workflow and operating environment that may reasonably be expected to occur with untrained operators in the intended use CLIA-waived setting. Test conditions were designed based on a risk analysis of the complete test system and included conditions intended to verify the effectiveness of built-in controls, lock-out features, and failure alerts.

To perform these studies, contrived samples were prepared using four (4) negative venous whole blood (VWB) samples and four (4) contrived HCV-positive samples (genotype 1a) prepared at 3X Limit of Detection (LoD) of the assay. Testing was conducted according to the package insert for the Xpert HCV test, except for the noted deviations dictated by the flex studies under evaluation. Results from each of the flex studies were compared to the respective control condition in which the sample and cartridge were prepared correctly for testing.

If such testing demonstrated activation of an appropriate fail-safe condition or failure alert mechanism, the associated engineering controls were determined to be effective, and no additional testing was performed. All flex conditions were evaluated using one lot of reagents (cartridges). Nine categories of potential sources of error were tested as listed in Table 4 below. Detailed description of the flex studies is presented below. In most cases, the expected positive or negative results were generated for each test condition, or the built-in fail-safe mechanisms or failure alerts were shown to function as intended to prevent reporting of erroneous results. No false results were obtained.

Table 4. List of Flex Studies for the Xpert HCV Test.

Category	Description
1	F1_Flex Study Incorrect Handling (Mixing) of Sample
2	F2_Flex Study Incorrect Handling (Mixing) of External Control
3	F3_Flex Study Incorrect Timing of Cartridge Preparation
4	F4_Flex Study Incorrect Test Volume
5	F5_Flex Study Incorrect Handling of Cartridge
6	F6_Flex Study High Heat and High Humidity
7	F7_Flex Study Low Heat and High Humidity
8	F8_Flex Study High Heat Low Humidity
9	F9_Flex Study Low Heat Low Humidity

The following flex studies were conducted previously and may be referenced in the CLIA Waiver Decision Summary for CW230013:

a. Operation of the instrument on non-level surface (instrument tilt)
b. When user attempted to use the GeneXpert instrument with improper ventilation
c. Stopping the test before completion by unplugging the instrument followed by restoration of power
d. Stopping the test by using the “STOP TEST” software icon on the GeneXpert Xpress screen
e. Stopping the test before the test is completed on the instrument then resume test using the same cartridge
f. Prepare sample and test on the GeneXpert Xpress instrument that is currently past its performance verification period
g. When user navigates the system’s touchscreen interface with two pairs of gloves
h. Incorrect handling of test cartridge:
    i. When cartridge is dropped before adding sample

ii. When cartridge is knocked before adding sample
iii. When cartridge is shaken three times before adding sample
iv. When cartridge reaction tube is missing
v. When cartridge reaction tube is broken
vi. When cartridge reaction tube is touched with and without gloves prior to starting a test
vii. When sample ID/patient ID label is placed on top of the cartridge lid blocking the plunger
viii. When cartridge lid is not fully closed after adding sample
ix. When user attempted to run the test with an expired cartridge
x. When user attempted to run the test with a spent (already used) cartridge

i. When user attempted to run the test with no sample added to cartridge
j. when the assay-specific definition file (ADF) was not loaded onto the GeneXpert Xpress system.

Flex Study F1 – Incorrect Handling (Mixing) of Samples
The test procedure for the Xpert HCV assay instructs the operator to mix the sample by gently inverting the tube 10 times before transferring to the cartridge. If the sample has been sitting in the microtainer for more than 15 minutes, the operator is instructed to gently invert the tube 20 times. This study evaluated the effect of improper handling (i.e., mixing) of samples in the microtainer. Simulated specimens in the microtainer were subjected to various episodes of vigorous manual mixing (5, 15, 20 times, as well as to the point of bubble production) and no mixing. In other conditions, samples were processed after sitting in the microtainer for 15 minutes and were subjected to various episodes of vigorous manual mixing (5, 10, 30 times) and no mixing. All testing conditions generated the expected results. To mitigate the risk of incorrectly handling of samples prior to addition to the test cartridge, sample preparation instructions are provided in the Xpert HCV QRI and package insert. In addition, the enclosed video shows how to perform “gentle mixing” of samples and external controls.

Flex Study F2 – Incorrect Handling (Mixing) of External Controls
The test procedure for the Xpert HCV assay instructs the operator to mix the external controls by gently inverting the tube 20 times. This study evaluated the effect of improper handling (i.e., mixing) of external controls. External positive and negative controls were subjected to various episodes of incorrect mixing (10, 30 times as well as to the point of bubble production), and no mixing at all. Incorrect handling (mixing) of the external controls did not result in non-determinant (ND) or erroneous results.

Flex Study F3 – Incorrect Timing of Cartridge Preparation
The test procedure for the Xpert HCV assay instructs the operator to immediately perform the assay following sample addition to the test cartridge. However, in some situations the testing could be delayed due to workload factors. Although specimen stability was demonstrated up to 4 hours at 2-30°C (specimen placed in collection tube), this study evaluated the risk of erroneous results when prepared specimens remained inside the test cartridge for different time intervals (15 and 30 minutes) prior to testing. Incorrect timing of cartridge preparation did not result in non-determinant (ND) or erroneous results. Based on sFMEA (safety Failure Modes and Effects Analysis), the potential erroneous results are mitigated by providing sample testing

instructions in the Xpert HCV QRI and package insert to test a cartridge immediately after adding sample to the cartridge.

Flex Study F4 – Incorrect Test Volume

The test procedure for the Xpert HCV assay instructs the operator to use the provided, fixed volume disposable transfer pipette (100 µL) to load the sample into the test cartridge. This study evaluated the potential for erroneous results when the operator does not follow the instructions and adds a range of sample volumes either below (25 µL, 50 µL, and 75 µL) or above (150 µL, 250 µL, and 500 µL) the expected volume. When an insufficient amount of sample was loaded (25 µL and 50 µL) to the sample chamber of the test cartridge, all positive and negative samples for each condition triggered fail-safe and failure alert mechanisms reported as "Error due to sample volume adequacy (SVA) termination (Error 2097)" and all cartridges resulted in non-determinant results. Additionally, when the cartridge was overloaded with 500 µL sample, all four (4) of the VWB negative samples also triggered fail-safe and failure alert mechanisms as expected and returned "NO RESULT – REPEAT TEST" results, yielding non-determinant results, while one of the 4 positive replicates returned "NO RESULT – REPEAT TEST" result. One additional replicate was run, and all valid results were reported.

Based on sFMEA, the risks of loading incorrect sample volume are mitigated by providing a fixed-volume pipette in the assay kit as well as step-by-step instructions to the user in the Xpert HCV QRI and package insert. There are also warnings in both the QRI and package insert indicating that insufficient sample volume may generate non-determinant results.

Flex Study F5 – Incorrect Handling of Test Cartridge

This study evaluated scenarios where the test cartridge was mishandled after sample addition, along with the impact of using incorrectly stored (at -20°C or at 37°C) cartridges. Flex study results and fail-safe/failure alert mechanisms that functioned in this flex study to prevent erroneous results are summarized below.

- Adding sample to cartridge and then dropping the cartridge from a height of 1 meter resulted in cartridge errors for 2 out of 4 negative control samples due to "Sample Volume Adequacy (SVA) error (Error 2097)" after sample leaked from the cartridge during the drop. The QRI and package insert contain a warning to the user that the cartridge should not be used in testing if it is dropped after the addition of sample.
- Adding sample to cartridge and then knocking over the cartridge resulted in "cartridge integrity error (Error 2037)" for 1 out of 4 positive samples. One additional replicate was run, and result was valid. The QRI and package insert contain a warning to the user that knocking over the cartridge after adding sample may lead to invalid results.
- Adding sample to cartridge and then shaking cartridge three times did not lead to erroneous results. However, based on the sFMEA, the potential erroneous results are mitigated by a warning to not shake the cartridge after sample addition in QRI and package insert.
- Incorrectly storing the cartridges below the 2-28°C storage requirements (at -20°C for 8 hours) or above the storage requirement (at 37°C for 8 hours) did not lead to erroneous results. However, the QRI and package insert indicate storing the cartridge at 2-28°C.

Flex Studies F6, F7, F8 and F9 – Extreme Heat and Extreme Relative Humidity

The Xpert HCV test has operational specifications clearly stated in the package insert, namely that the testing should be performed at temperatures ranging between 15-30°C with relative humidity (RH) between 20% and 80%. This study evaluated the impact of operating the Xpert HCV test outside of the specified environmental conditions. A summary of the flex study results is provided below. As a control for each flex study, ambient laboratory condition was tested, with the acceptable temperature of 20-30°C and RH between 30% and 60%.

- During the high heat and high humidity (40°C and 95% RH) flex study 6 (F6), the GeneXpert Xpress instrument and the cartridges were exposed to heat and humidity conditions and external controls were tested before running the samples, to ensure that controls produced the expected results. External controls were unable to successfully run in the test environment due to instrument failure. The RH was lowered to 80% and the study was completed per protocol. All external controls and samples met the acceptance criteria in the environment (40°C and 80% RH).
- The low heat and high humidity (10°C and 95% RH) flex study 7 (F7) did not produce erroneous results.
- During the high heat and low humidity (40°C and <20% RH) flex study 8 (F8), two of the four positive replicates returned instrument error. Additional replicates were run and returned valid results.
- During the low heat and low humidity (10°C and <20% RH) flex study 9 (F9), one of the positive controls returned a NO RESULT – REPEAT TEST error, while the testing condition did not result in erroneous results.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Comparision Study

a. Study Design

i. Study Sites and Duration

Clinical performance characteristics of the Xpress HCV test were evaluated in a multi-site prospective study in fingerstick whole blood collected from individuals at-risk and/or with signs and symptoms of HCV infection. The study was conducted at 15 CLIA waived sites from geographically diverse locations in the United States. The sites included infectious disease clinics, drug use research clinics, emergency departments and harm reduction clinics. All 15 sites qualified as representative of CLIA waived intended use sites for this device. One (1) additional site conducted the comparator testing. Study participant enrollment, specimen collection, and Xpert HCV testing began in February 2024 and concluded in May 2024.

ii. Operators

There were a total of 32 operators representative of intended CLIA waived users across the 15 clinical testing sites. The participating operators consisted of clinical research coordinators, phlebotomists, nurses, and medical assistants. The operators who participated in the clinical study were representative of operators in a CLIA waived setting and untrained in the use of the Xpert HCV test. Upon completion of the study, the operators at

each site were asked to complete an Operator Questionnaire that asked them to rate the ease of use of the test procedure.

iii. Instructions for Use

The operators were given the Xpert HCV package insert and the Quick Reference Guide. No other materials or instructions were provided and the operators received no training in the use of the test.

iv. Subjects (Patients)

A total of 1279 individuals were enrolled in the study. Specimens for the prospective clinical study were collected according to the following inclusion criteria:

- Participant was ≥18 years old
- Participant was not on treatment at time of enrollment based on review of medical records or self-reported
- Participant had signs and symptoms and/or is considered at-risk of HCV infection
- Participant is able to and agreed to provide the following specimens for study purposes:
  - 1 x ≥250µL fingerstick whole blood (CWB) specimen in a microtainer containing K_2EDTA
  - 2 x ≥ 5mL venous whole blood (VWB) specimen in Serum Separator Tubes (SST)
  - An additional one (1) ≥ 5mL VWB in K_2EDTA tube, if participant consents.
- Provided documented informed consent or assent as required by the reviewing institutional review board (IRB). Documented receipt of the Experimental Bill of Rights for all study participants enrolled in applicable states.

The exclusion criteria for the study were as follows:

- Clinician assessed that the participant was not suitable for inclusion e.g., the participant was on treatment at the time of enrollment, had no signs or symptoms of hepatitis, or had no risk factor for HCV.
- Participant was previously enrolled in the study.
- CWB specimens were not collected according to the manufacturer's instructions.

v. Samples

A total of 1,279 individuals were enrolled in the study and samples from a total of 1,012 individuals were included in the study. Of the 1,012 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); 3) non-evaluable comparator test results (n=4); A total of 982 samples tested were considered evaluable for the purpose of data analysis in the accuracy study.

vi. Comparative Method (CM)

The comparator was based on a patient infected status (PIS) classification which includes results from both the FDA-approved HCV RNA test and FDA-approved HCV antibody test. The four categories of PIS classification are listed in Table 5 below.

Table 5. Categories of Patient Infected Status Classification

HCV Antibody Test	HCV RNA Test	PIS Classification
Reactive	HCV detected	Active Chronic Infection (HCV Positive)
	HCV not detected	Past/Resolved Infection (HCV Negative)
Non-Reactive	HCV detected	Active Acute Infection (HCV Positive)
	HCV not detected	Not Infected (HCV Negative)

b. Results and Analysis

i. Statistical Analysis of Comparison Study Results

Table 6 presents the Xpert HCV test results by patient infected status (PIS) included in the performance calculation.

Table 6. Summary of samples by Patient Infected Status (PIS)

Group	HCV Antibody Test	HCV RNA Test	PIS Classification	Xpert HCV	N
I	Reactive	Detected	Active Chronic Infection	HCV DETECTED	111
				HCV NOT DETECTED	6
II		Not detected	Past/resolved infection	HCV DETECTED	1
				HCV NOT DETECTED	223
III	Non-reactive	Detected	Active Acute Infection	HCV DETECTED	3
				HCV NOT	2

Group	HCV Antibody Test	HCV RNA Test	PIS Classification	Xpert HCV	N
				DETECTED	
IV		Not detected	Not Infected	HCV DETECTED	1
				HCV NOT DETECTED	635

Table 7 summarizes the Xpert HCV Test performance relative to the PIS when used by untrained operators. The Xpert HCV test demonstrated positive percent agreement (PPA) and negative percent agreement (NPA) of 93.44% and 99.77%, respectively when compared to the PIS (Table 7).

Table 7. Clinical performance of the Xpert HCV Test according to Patient Infected Status (PIS)

		Patient Infected Status		
		HCV Positive[a]	**HCV Negative[b]**	**Total**
Xpert HCV Test	HCV DETECTED	114	2*	116
	HCV NOT DETECTED	8*	858	866
	Total	122	860	982
PPA (114/122) 93.44% (95% CI: 87.59 - 96.64)				
NPA (858/860) 99.77% (95% CI: 99.16 - 99.94)				

PPA; Positive Percent Agreement, NPA; Negative Percent Agreement, 95% CI; 95% score confidence interval

[a] Active chronic or acute infection.

[b] Past/resolved infection or not infected.

*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.

Of the 1,012 Xpert HCV runs performed in the clinical study, 61 resulted in non-determinant ("INSTRUMENT ERROR" or "NO RESULT - REPEAT TEST") results on first attempt. Upon retest of these 61 specimens, 12 remained non-determinant. The initial non-determinate rate was 6.0 % (61/1,012) and the overall non-determinate rate was 1.2 % (12/1,012).

ii. Device Performance with Analyte Concentrations Near the Limit of Detection (LoD)

The performance of the Xpert HCV Test with samples at HCV concentration relative to the assay Limit of Detection (LoD) was evaluated with a reproducibility study conducted in support of the De Novo for this device. 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). Six HCV positive panel members were prepared by contriving pooled HCV negative venous whole blood with HCV clinical strains from genotype (GT) 1a, GT 2b and GT 3a to target levels shown in Table 8 below.

Table 8. Reproducibility Panel for the Xpert HCV test

Panel Member	Target	Actual Concentration, IU/mL	Level
1	Negative[a]	0	Negative
2	HCV clinical strain (GT1a)[b]	129.3	1.5x LoD
3	HCV clinical strain (GT1a)[b]	258.7	3.0x LoD
4	HCV clinical strain (GT2b)	100.1	1.5x LoD
5	HCV clinical strain (GT2b)	200.2	3.0x LoD
6	HCV clinical strain (GT3a)	75.7	1.5x LoD
7	HCV clinical strain (GT3a)	151.3	3.0x LoD

[a] "Negative" indicates that the sample does not contain any target microorganism or target RNA.
[b] Panel member prepared with the 6th WHO International Standard (IS) that is manufactured from HCV positive human plasma.

Nine untrained users (3 operators per site) at the CLIA waived sites participated in the study. The study was conducted 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 or higher) at 3 sites. The total number of replicates per sample tested was 90. The results of testing of the Xpert HCV Test with samples near the assay LoD stratified by operator and site are summarized in Table 9 below. In summary:

- Out of 630 samples tested, no invalid test results were obtained.
- Negative samples were called negative 98.9% (89/90) of the times.
- Low Positive samples (1.5x LoD) were called positive 98.9% (89/90) of the times for HCV GT1a, 96.7% (87/90) for GT2b, and 100% (90/90) for GT3a.

- Moderate Positive samples (3.0x LoD) were called positive 98.9% (89/90) of the times for HCV GT1a, and 100% (90/90) for GT2b and GT3a.
- There were no significant differences in observed positivity of the device with low positive samples between operators or between sites.

Table 9. Performance of the Xpert HCV Test with Samples Near the Assay LoD by Untrained Operators

Panel Member	Site 1				Site 2				Site 3				Overall Agreement and 95% CI
	Op 1	Op 2	Op 3	Subtotal	Op 1	Op 2	Op 3	Subtotal	Op 1	Op 2	Op 3	Subtotal	
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% (89/90) 94.0% –99.8%
GT1a 1.5x LoD	100% (10/10)	90% (9/10)	100% (10/10)	96.7% (29/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	98.9% (89/90) 94.0% –99.8%
GT1a 3.0x 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% (89/90) 94.0% –99.8%
GT2b 1.5x 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% (87/90) 90.7% –98.9%
GT2b 3.0x 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% (90/90) 95.9% – 100%
GT3a 1.5x 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% (90/90) 95.9% – 100%
GT3a 3.0x 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% (90/90) 95.9% – 100%

Abbreviation: Op, operator

The study results demonstrated that users untrained in the test procedure of the Xpert HCV test were able to perform the test correctly and the test provided the expected result for samples near the LoD.

2. Operator Questionnaire

A user questionnaire was administered to the participating operators at the conclusion of the clinical study to obtain feedback on the ease of use of the Xpert HCV test and the GeneXpert Xpress instrument. The questionnaire consisted of 21 questions (statements) divided into three distinct categories: 1) GeneXpert Xpress System set-up (3 statements),

2) system operation and performing the Xpert HCV test (14 statements), and test result interpretation (3 screenshots with results). Of 31 operators who participated in the clinical and/or reproducibility/near cut-off studies for the Xpert HCV assay, all completed the post-study user questionnaire, 24 of whom only participated to the Xpert HCV clinical study and 7 participated in both the Xpert HCV clinical and reproducibility studies. Question 6 "I have experience working in a moderately complex lab environment" was answered "No" by 100% (31/31) of users.
Majority of the users answered either "Strongly Agree" or "Agree" to the 3 statements related to System set-up. In addition, majority of the users (30/31) answered either "Strongly Agree" or "Agree" that it was easy to understand instructions from QRI, video, to invert the tube, transfer loaded cartridge to the instrument and what to do with the cartridge after the test was complete. Majority of the operators (25/31) found the pipette to transfer the sample from the tube to the cartridge easy to use. All users (31/31) answered either "Strongly Agree" or "Agree" that it was easy to understand the results of the test. Overall, all users agreed that it was easy to perform the Xpert HCV test.
When operators were presented with three different screenshots of possible Xpert HCV test results, all operators but one (30/31) interpreted result correctly for 2 scenarios and all operators interpreted results correctly for the third scenario (31/31).

Overall, based on the operators' responses, the Xpert HCV test on the GeneXpert Xpress System was easy to use and the materials provided (Getting Started Guide and Quick Reference Instructions) were adequate to assemble the system and perform the test without additional instruction.

M. Labeling for Waived Devices

a. The labeling for the Xpert HCV test consists of:

1. Xpert HCV Package Insert
2. Xpert HCV Quick Reference Instructions
3. GeneXpert Xpress System User's Guide (Operator Manual)
4. GeneXpert Xpress System Getting Started Guide

b. <u>The following elements are appropriately present:</u>

- The GeneXpert Xpress System User's Guide, Xpert HCV package insert, and Xpert HCV Quick Reference Guide specify the environmental operating conditions under which testing may be performed.
- The GeneXpert Xpress System User's Guide, GeneXpert Xpress System Getting Started Guide, and the Xpert HCV QRI are clear, easy to understand and, where appropriate, contain graphic representation of system components and procedure steps.
- The Xpert HCV package insert and the Xpert HCV QRI:
    - Identify the test as CLIA-waived and contain a statement that a Certificate of Waiver is required to perform the test in a waived setting; information on how operators can obtain a certificate is also provided.

    - Indicate that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 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.
    - Include step-by-step instructions for performing the test with clinical specimens.
    - Include safety considerations applicable for untrained users.
    - Specify the actions to be taken if a non-determinant test result (INSTRUMENT ERROR or NO RESULT – REPEAT TEST) is obtained.
    - Include appropriate warnings and/or limitations pertaining to clinical interpretation of test results.
    - Include recommendations for Quality Control testing, including the source of appropriate control materials and the frequency of testing.
- The Xpert HCV package insert includes a summary of the studies performed to support CLIA Waiver.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Benefit-Risk Considerations

Not applicable.

O. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.