EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Xpert HCV; GeneXpert Xpress System
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN240016

B Applicant

Cepheid

C Proprietary and Established Names

Xpert HCV; GeneXpert Xpress System

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SBP	Class II	21 CFR 866.3171 - Simple point-of-care nucleic acid-based hepatitis C virus ribonucleic acid test	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for Xpert HCV test and GeneXpert Xpress System.

B Measurand:

Hepatitis C Virus RNA in human fingerstick whole blood.

C Type of Test:

Automated qualitative reverse transcription polymerase chain reaction (RT-PCR).

III Indications for Use:

A Intended Use(s):

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 K2-EDTA 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.

B Indication(s) for Use:

See Intended Use above.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

GeneXpert Xpress System

IV Device/System Characteristics:

A Device Description:

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 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 (Hub configuration) consists of a GeneXpert IV instrument that conducts 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 (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), 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 K2-fingerstick EDTA whole blood. The BD Microtainer for capillary whole blood collection was validated for use with the Xpert HCV test. After collecting human fingerstick EDTA 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.

Materials Provided in the Xpert HCV Test:
The Xpert HCV test Kit contains sufficient cartridges to process 10 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 (For use with the GeneXpert Xpress System)	**1 per kit**
Quick Reference Instructions (For use with the GeneXpert Xpress System)	**1 per kit**
CD	**1 per kit**
• Assay Definition File (ADF)	
• Instructions to import ADF into GenXpert Xpress System	

Material required but not provided with the kit:

- GeneXpert Xpress System (catalog number: GXIV-2-CLIA or GXIV-4-CLIA): 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). Please note that sample collection might be difficult in individuals with callused fingers, and a deeper lancet might be needed.
- EDTA-containing capillary collection tubes for small volumes (K2 EDTA microtainer BD part number: 365974).

- NAT troll Hepatitis C Virus Positive and Negative controls
- Alcohol wipes
- Gauze pad
- Bandage
- Warm pack

B Principle of Operation

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. 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.

Table 1. Xpert HCV test Results and interpretation.

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 the sample

C Instrument Description Information

1. Instrument Name:

   The Xpert HCV test is performed on the Gene Xpert Xpress System (Hub Configuration) with GeneXpert Xpress Software Version 6.4a or higher.

   The GeneXpert Xpress System (Hub configuration) consists of a GeneXpert IV instrument with the capability of running 2 or 4 modules and a single-unit accessory called the GeneXpert Hub that integrates the computer, touchscreen monitor and barcode scanner. The

GeneXpert Xpress System contains preloaded GeneXpert Xpress software for running the tests and viewing the results (executes the sample preparation, nucleic acid amplification, and real-time fluorescent signal detection for the test). Simple instructions (GeneXpert Xpress *Getting Started Guide*) are provided for attaching the Hub and the GeneXpert System. The system is capable of bi-directional connectivity (upload and download) with the Laboratory Information System (LIS).

The GeneXpert module is the basic functional operation unit common to all GeneXpert Systems including the GeneXpert Xpress System. Each GeneXpert module is identical and operates independently. Each module consists of a syringe pump drive for dispensing fluids, an ultrasonic horn for lysing cells or spores, a valve drive to rotate the cartridge valve body for sample movement across the different cartridge chambers, and a proprietary microprocessor controlled I-CORE (Intelligent Cooling/Heating Optical Reaction) thermocycler for performing real-time PCR amplification and fluorescence signal detection.

2. Specimen Identification:

To perform a test, the user touches the 'NEW TEST' icon on the Home Screen. Either patient information must be entered if configured by an administrator or the Sample ID screen appears.

- If the Patient Information screen appears, the operator manually enters the patient ID or scans the patient ID barcode for the patient's specimen.
- If the Sample ID screen appears, the operator scans the sample ID barcode or manually enter the Sample ID for the patient specimen.

The user is then instructed to scan the cartridge barcode and confirm that the appropriate cartridge for the Xpert Xpress HCV test has been selected. The test cartridge is prepared and loaded into an available instrument module that has a flashing green light which initiates the test.

3. Specimen Sampling and Handling:

The Xpert HCV test is designed for use with human K2- EDTA fingerstick whole blood. The BD Microtainer for capillary whole blood collection was validated for use with the Xpert HCV test. The fingerstick whole blood collection should follow the package insert provided in the BD Microtainer kit. In addition, the fingerstick whole blood collection instructions are also provided in the Xpert HCV package insert, and a fingerstick whole blood collection instruction video can be accessed by scanning the QR code provided in the Xpert HCV package insert and Quick Reference Instructions (QRI). This video must be viewed before collecting the first patient sample. The video should be viewed again if sample collection issues are experienced.

4. Calibration:

Cepheid performs all necessary optical and thermal calibrations of the GeneXpert Xpress instrument at the time of manufacture, prior to customer installation; therefore, calibration of the instrument is not required during initial system startup. Cepheid recommends that the system be checked for proper calibration on an annual basis from the point of initial use. A

GeneXpert operator or Cepheid Field Service Engineer with Administrator user permissions can perform calibration checks during annual maintenance.

5. Quality Control:
   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 TESTING 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. For SPC, the valid Ct range is 31.0 to 37.0. For IC-H, the valid Ct range is 20.0 to 26.0.

   - 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.

   External Controls:
   Additionally, external positive and negative quality controls are available but not provided with the kit:
   - NAT troll Hepatitis C Virus Positive Control (ZeptoMetrix Corporation, NATHCV-6C-IVD).
   - NAT troll Hepatitis C Virus Negative Control (ZeptoMetrix Corporation, NATHCVNEG-6C-IVD).

V Standards/Guidance Documents Referenced:

Standards

CLSI EP05-A3. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

CLSI EP07-3rd Ed. Interference Testing in Clinical Chemistry, 3rd Edition. CLSI guideline EP07. Wayne, PA. Clinical and Laboratory Standards Institute; 2018.

CLSI EP09c- 3rd Ed. Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd Ed. CLSI guideline EP09c. Wayne, PA. Clinical and Laboratory Standards Institute; 2018.

CLSI EP12-A2. User Protocol for Evaluation of Qualitative Test Performance, Approved Guidance – Second edition. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

CLAI EP 17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline – 2nd Edition. CLSI document EP17-A2. Clinical and Laboratory Standards Institute; 2012.

CLSI EP25-A. Evaluation of Stability of In Vitro Medical Laboratory Test Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

CLSI MM03 3rd Ed. Molecular Diagnostic Methods for Infectious Diseases, 3rd Edition. CLSI document MM03. Clinical and Laboratory Standards Institute; 2015.

CLSI MM13 2nd Ed. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods, 2nd Edition. CLSI guideline MM13. Clinical and Laboratory Standards Institute; 2020.

ISO 14971:2019-12. Medical Devices – Application of Risk Management to Medical Devices. Third Edition.

ISO 13485:2016. Medical devices - Quality management systems - Requirements for regulatory purposes.

ANSI AAMI IEC 62304:2006/A1:2016: Medical device software - Software life cycle processes.

IEC 60601-1-2, Edition 4.1 2020-09. Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests.

IEC 61326-1, Edition 3.0 2020-10. Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements.

IEC 61326-2-6, Edition 3.0 2020-10. Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment.

Guidance documents

Acceptance Review for De Novo Classification Requests: Guidance for Industry and Food and Drug Administration Staff, issued October 5, 2021.

De Novo Classification Process (Evaluation of Automatic Class III Designation): Guidance for Industry and Food and Drug Administration Staff, issued October 5, 2021.

Consideration of Uncertainty in Making Benefit-Risk Determinations in Medical Device Premarket Approvals, De Novo Classifications, and Humanitarian Device Exemptions: Guidance for Industry and Food and Drug Administration Staff, issued August 30, 2019.

Factors to Consider When Making Benefit-Risk Determinations in Medical Device Premarket Approval and De Novo Classifications: Guidance for Industry and Food and Drug Administration Staff, issued August 30, 2019.

eCopy Program for Medical Device Submissions: Guidance for Industry and Food and Drug Administration Staff, issued April 27, 2020.

Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices: Guidance for Industry and Food and Drug Administration Staff, issued September 14, 2018.

Off-The-Shelf Software Use in Medical Devices: Guidance for Industry and Food and Drug Administration Staff, issued August 11, 2023.

Content of Premarket Submissions for Device Software Functions: Guidance for Industry and Food and Drug Administration Staff, issued June 14, 2023.

General Principles of Software Validation: Guidance for Industry and FDA Staff, issued January 11, 2002.

Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions: Guidance for Industry and Food and Drug Administration Staff, issued on September 27, 2023.

Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software: Guidance for Industry, issued January 14, 2005.

Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable: Guidance for Sponsors, Institutional Review Boards, and Food and Drug Administration Staff, issued April 25, 2006.

Electromagnetic Compatibility (EMC) of Medical Devices: Guidance for Industry and Food and Drug Administration Staff, issued June 6, 2022.

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

Precision Study: 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, during 5 independent days of testing, 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 or higher). The total number of replicates per sample tested was 60. Table 2 summarizes the percent correct for each panel level and 95% CI using the Wilson score method.

Table 2. 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) samples yielded non-determinate results on initial and 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-run, between-days, between-lots, and total precision for each panel member are presented in Table 3.

Table 3. 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

Multi-Site Reproducibility Study: 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 or higher). The total number of replicates per sample tested was 90. Table 4 summarizes the percent correct for each panel level, and 95% CI using the Wilson score method.

Table 4. Summary of Reproducibility Percent Agreement Results for the Xpert HCV test.

Target and Level	Site 1				Site 2				Site 3				Agreement by Target
	Op1	Op2	Op3	Site Total	Op1	Op2	Op3	Site Total	Op1	Op2	Op3	Site Total	(%; 95%CI)
Negative	100%	100%	90%	96.7%	100%	100%	100%	100%	100%	100%	100%	100%	98.9%
													(94.0% - 99.8%)
	10/10	10/10	9/10	29/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	89/90
GT1a 1.5 x LoD	100%	90%	100%	96.7%	100%	100%	100%	100%	100%	100%	100%	100%	98.9%
													(94.0% - 99.8%)
	10/10	9/10	10/10	29/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	89/90
GT1a 3.0 x LoD	100%	100%	90%	96.7%	100%	100%	100%	100%	100%	100%	100%	100%	98.9%
													(94.0% - 99.8%)
	10/10	10/10	9/10	29/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	89/90
GT2b 1.5 x LoD	100%	90%	100%	96.7%	100%	90%	100%	96.7%	100%	90%	100%	96.7%	96.7%
													(90.7% - 98.9%)
	10/10	9/10	10/10	29/30	10/10	9/10	10/10	29/30	10/10	9/10	10/10	29/30	87/90
GT2b 3.0 x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
													(95.9% - 100%)
	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	90/90
GT3a 1.5 x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
													(95.9% - 100%)
	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	90/90
GT3a 3.0 x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
													(95.9% - 100%)
	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	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) are provided for each covariate: within-run (repeatability), between-operators, between-days, between-sites, and total reproducibility for each panel member are presented in Table 5.

Table 5. 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
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

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

2. Linearity:

Not Applicable.[1]

3. Analytical Specificity/Interference:

Analytical Specificity: The analytical specificity of the Xpert HCV test was evaluated by testing a panel of 27 organisms that can be encountered in samples drawn for HCV testing. These organisms were tested at a concentration of 100,000 CFU/mL, copies/mL, or TCID50/mL as applicable. Each organism in the panel was tested in the presence (3-time Limit of Detection (LoD)) and absence of HCV RNA in replicates of 6. Positive and Negative HCV controls tested in replicates of 6 were also included in the study. The study was completed using a single kit lot.

None of the organisms tested in this study (Table 6) showed cross reactivity with the HCV detection using the Xpert HCV test in HCV-positive and HCV-negative samples.

Table 6. List of organisms tested in the analytical specificity study.

Virus	Bacteria	Fungus
Human Immunodeficiency virus 1 (HIV-1) Human Immunodeficiency virus 2 (HIV-2) 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) Herpes simplex Virus 6 (HHV-6) Herpes simplex Virus 8 (HHV-8) Varicella Zoster Virus (VZV) BK Human Polyoma Virus	*Staphylococcus aureus* *Streptococcus epidermidis*	*Candida albicans*

[1] Linearity study is not applicable to Xpert HCV test because the device is qualitative. Special control (b) (5) (i) applies to quantitative devices only.

Human papilloma Virus 16 (HPV-16) Human papilloma Virus 18 (HPV-18) St. Louis Encephalitis Virus Vaccinia Virus		

Interference Study: The Xpert HCV test was evaluated for interference in the presence of elevated levels of endogenous and exogenous substances commonly found in fingerstick whole blood specimens from individuals in the intended use population. 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 endogenous substance consisting of HCV negative EDTA venous whole blood and HCV negative EDTA venous whole blood spiked with HCV to 300 IU/ml was included and tested in replicates of six. This study was conducted using one kit lot.

None of the substances and autoimmune conditions tested in this study interfere with the Xpert HCV test at the concentrations listed in the tables below.

Table 7. Endogenous interferents evaluated with the Xpert HCV test.

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 8. Exogenous interferent pools evaluated with the Xpert HCV test.

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
	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 9. Autoimmune disease samples evaluated with the Xpert HCV test.

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

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Fingerstick Whole Blood specimen stability: The stability of hepatitis C virus genotype 1a in fingerstick whole blood (FS) was evaluated after storage at 2°C and 30°C for up to five hours. Freshly drawn FS collected from 6 individuals were included in the study. Individuals providing the FS were confirmed HCV-negative using cobas HCV assay. Each individual provided three 250 – 500 µl samples of FS in K2 EDTA microtainer tubes. FS samples from five individual were spiked at a final concentration of 3x LoD with contrived HCV-positive venous whole blood (VWB) samples. FS samples from one individual were spiked with HCV-negative VWB to generate HCV-negative samples.

   Samples were stored at 2°C or 30°C and tested in singlicate at baseline (T=0 hours), 2 hours, 4 hours, and 5 hours. For each sample at each timepoint, test results were reported as HCV DETECTED and HCV NOT DETECTED.

The study results support the stability of FS stored for up to 4 hours at 2°C and 30°C.

External Controls: the external positive and negative controls are available but not provided with the Xpert HCV test. The external controls for use with the Xpert HCV test include:

- NAT troll Hepatitis C Virus Positive Control (ZeptoMetrix Corporation, NATHCV-6C-IVD).
- NAT troll Hepatitis C Virus Negative Control (ZeptoMetrix Corporation, NATHCVNEG-6C-IVD).

The instruction for use recommends that external controls be tested at the frequency noted 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.

6. Detection Limit:

The LoD of the Xpert HCV test was determined by testing dilutions of hepatitis C virus RNA for genotypes 1a, 1b, 2b, 3a, 4, 5 and 6 in HCV negative human K2-EDTA fingerstick whole blood. Each panel was tested following the package insert. Probit analysis was used to determine the LoD. Table 10 below summarizes the LoD for each genotype in fingerstick whole blood.

Table 10. Summary of Xpert HCV LoD 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)

7. Assay Cut-Off:

Not Applicable

8. Accuracy (Instrument):

Not Applicable

9. Carry-Over:

Not Applicable

B Comparison Studies:

1. Method Comparison:

   Not Applicable

2. Matrix Comparison:

   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

   Not Applicable

2. Clinical Specificity:

   Not Applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Prospective all-comers blinded clinical study was conducted to evaluate the performance of the Xpert HCV test. The study was conducted at 15 CLIA waived sites from geographically diverse locations in the US with 32 untrained operators. Participants included individuals ≥ 22 years of age who were at risk and/or with signs and symptoms for HCV infection. A total of 1,012 fingerstick whole blood (FS) specimens were obtained from eligible participants for testing with the Xpert HCV test. In addition, serum specimens were tested on an FDA approved HCV RNA test and an FDA approved HCV antibody test to establish the patient infected status (PIS). Patient management continued at the site per the standard practice, independent of investigational test results.

   Table 11 below summarizes the total number of participants by risk factor. Available demographic data collected from study participants are presented Table 12.

Table 11. Categorization of Risk Factors for the Eligible Participants

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.

Table 12. 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%)
	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%)
	NA	158 (15.6%)
	Never had an 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 an 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%)
	NA	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%)

Demographic Characteristics		Overall (N-=1012)
	1c	2 (0.2%)
	2b	2 (0.2%)
	3a	5 (0.5%)
	No Result[e]	967 (95.6%)
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 FS samples tested were included in the Xpert HCV test performance calculations. Table 13 summarizes the Xpert HCV test results by patient infected status (PIS).

Table 13. Summary of samples by infected status (PIS)

HCV Ab	HCV RNA NAAT	Patient Infection 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 DETECTED	635

The Xpert HCV test performance was compared to a patient infected status (PIS) algorithm based on results from an FDA approved HCV RNA test and an antibody test. Table 14 below summarize the Xpert HCV test performance. 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 14. Xpert HCV test result according to PIS

		Patient Infection 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*		93.44%; 95% CI (87.59% – 96.64%)		
NPA*		99.77%; 95% CI (99.16% – 99.94%)		

[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.

4. Non-Determinate Rate

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

5. Testing non-viral hepatitis samples

FS 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.

The table below shows subject demographics and liver disease status for the 78 evaluable subjects.

Table 15: 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	N/A	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	N/A	100.0% (95% CI: 34.2 - 100.0)
Alcoholic liver disease	11	10	0	0	10	0	N/A	100.0% (95% CI: 72.2 - 100.0)
Autoimmune hepatitis	3	3	0	0	3	0	N/A	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

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

Not Applicable

F Other Supportive Performance Characteristics Data:

Not Applicable

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Identified Risks to Health	Mitigation Measures
Risks of false test results	Certain labeling information, including limitations, device descriptions, explanation of procedures, and results interpretation information. Certain design verification and validation information, certain analytical studies and clinical studies, risk analysis strategies, lot release criteria, flex studies, and stability studies.
Failure to correctly interpret the results	Certain labeling information, including limitations, device descriptions, explanation of procedures, and results interpretation information. Certain design verification and validation information, including critical reagent information, risk analysis strategies, lot release criteria, flex studies, and stability studies and protocols.
Failure to correctly operate the device	Certain labeling information, including limitations, device descriptions, and explanation of procedures. Certain design verification and validation information including critical reagent information, risk analysis strategies, lot release criteria, flex studies, and stability studies.

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The benefit of the assay is the ability to detect HCV RNA to aid a clinician in the diagnosis of hepatitis C virus (HCV) infection in individuals with signs and symptoms or risk factors for HCV infection without needing an HCV antibody result. Diagnosis of HCV infection with the aid of this device can occur within one encounter with the healthcare system. Individuals diagnosed with HCV infection with the aid of the candidate device can be treated with indicated antiviral treatment. Treatment of HCV infection helps prevent known complications of undiagnosed and untreated HCV infection, including by symptom alleviation, decreases in all-cause mortality, liver disease-related complications and death, hepatocellular carcinoma rates, and need for liver transplantation. Diagnosis of HCV infection can improve patient knowledge of the condition, inform prognosis, and guide the need for additional imaging or laboratory testing. Diagnosis of HCV infection also leads clinicians to evaluate and subsequently treat patients for human immunodeficiency virus (HIV) and hepatitis B virus (HBV), if indicated, as these viruses share common modes of transmission with HCV, and co-infection is widely documented.

Diagnosis could also decrease community transmission among other individuals at risk for HCV infection.

B Summary of the Assessment of Risk:

The risks associated with the device, when used as intended, are those related to the risk of false test results (false positive and false negative), failure to correctly interpret the test results, and failure to correctly operate the instrument.

Risks of a false positive test include unnecessary imaging, laboratory testing, and treatment, repeating hepatitis C testing and additional imaging and laboratory evaluations. False positive test results may have psychosocial implications for those tested.

Risks of false negative test results include missed diagnosis of HCV infection. Risks of a false negative test include the failure to treat HCV infection. Failure to diagnose and treat HCV infection will lead to increased all-cause mortality and increased likelihood of liver disease-related complications, including cirrhosis, hepatocellular carcinoma, and the need for liver transplantation. A failure to diagnose HCV infection could also lead to increased community transmission and disease burden in individuals at risk for infection. A false negative result may deny a clinician the opportunity to evaluate and treat a patient who is co-infected with HIV or HBV, as these viruses share common modes of transmission with HCV, and patients are often co-infected. Additional risks of false negative test results are unnecessary additional diagnostic procedures in pursuit of a different cause for the patient's hepatitis, which bear known risks such as bleeding and infection for liver biopsies and pharmacological treatment for other etiologies, which have known adverse effects such as hypersensitivity reactions.

Failure to correctly operate the device could lead to invalid results requiring retesting and leading to delayed test results. The risk of a delayed result is minimal, given the short time to results with the device. Failure to correctly interpret results carries the same risks as false results.

C Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D Summary of the Assessment of Benefit-Risk:

The risks associated with the device (risk of false test results, failure to correctly interpret the results, and failure to correctly operate the device) are mitigated by labeling information, which will assist the operator in correctly performing the test and will assist healthcare providers in understanding the intended use of the test and evaluating the predictive value of a result based on the analytical and clinical performance of the test. In addition, the risks of false test results and failure to correctly interpret the results are mitigated by certain design verification and validation activities, including analytical and clinical studies and risk analysis strategies to reduce the likelihood of such errors. Such measures help to ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results. In addition, the device's performance observed in the clinical study suggests that errors will be uncommon and that the assay will provide substantial benefits to patients as an aid in the diagnosis of HCV infection in individuals at risk and/or with signs and symptoms of HCV infection with or without antibodies to HCV. There is significant morbidity and mortality associated with the natural history of disease progression of undiagnosed HCV, and barriers exist to initiation of antiviral treatment due to loss-to-follow up with current multi-step HCV testing algorithms over multiple encounters with the healthcare system. Because this test can be used at the point of care and can return a

result in approximately 60 minutes, the diagnosis of HCV can occur in one encounter with the healthcare system. Risks can be further mitigated by labeling the device as "prescription use only" and by including additional warnings noting limitations of safety information, including populations or situations for which the safety and efficacy of the device has not been evaluated. While general controls alone are insufficient to mitigate the risks associated with the device, given the special controls, the benefits outweigh the risks.

X Conclusion:

The De Novo request is granted, and the device is classified under the following, and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s):	SBP
Device Type:	Simple point-of-care nucleic acid-based hepatitis C virus ribonucleic acid test
Class:	Class II
Regulation:	21 CFR 866.3171