CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW260013

II. Parent Document Number

K261362

III. CLIA Waiver Type

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

IV. Applicant

Cepheid

V. Proprietary and Established Names

Xpert Hemorrhagic Fever

VI. Measurand (analyte)

The assay detects and identifies nucleic acids of the following organisms: Ebola virus (including Zaire, Sudan, Taï Forest, and Bundibugyo, not differentiated)[1], Crimean-Congo hemorrhagic fever (CCHF) virus, Marburg virus, and Lassa virus.

VII. Sample Type(s)

EDTA-treated whole blood specimens collected by capillary or venous draw

VIII. Type of Test

Multiplex Nucleic Acid Amplification Test

IX. Test System Description

[1] The nomenclature of the family *Filoviridae*, including the viruses that cause Ebola disease, has changed over time. Recently, the genus name *Ebolavirus* was changed to *Orthoebolavirus*, and the species name Zaire ebolavirus was changed to *Orthoebolavirus zairense*. Refer to: *Biedenkopf N., et al, Renaming of genera Ebolavirus and Marburgvirus to Orthoebolavirus and Orthomarburgvirus, respectively, and introduction of binomial species names within family Filoviridae. Arch Virol., 2023 Aug 3;168(8):220. doi: 10.1007/s00705-023-05834-2. PMID: 37537381*

A Overview

The Xpert Hemorrhagic Fever (Xpert HF) test is an automated *in vitro* diagnostic test for the qualitative detection and identification of RNA from Ebola virus (including strains Zaire, Sudan, Taï Forest, and Bundibugyo, not differentiated), CCHF virus, Marburg virus, and Lassa virus in EDTA-treated whole blood specimens collected by capillary or venous draw from individuals with signs and symptoms of the suspected infections and/or individuals who are at risk for exposure or may have been exposed to these agents.

The Xpert HF test is intended for use only by U.S. Department of Defense laboratories having appropriate personal protective equipment (PPE) and other safety precautions including personnel trained in the safe handling of diagnostic clinical specimens potentially containing highly infectious agents. The test is for prescription use only. The test is performed on the GeneXpert Edge X system. With this platform, an untrained operator can perform the test in four steps: 1) add EDTA-treated whole blood specimens to off-board sample reagent vial (using transfer pipets and reagent vials provided in the test kit) for inactivation, 2) transfer inactivated blood sample to the test cartridge with a transfer pipette (also provided in the test kit), 3) run the test on the GeneXpert Edge X system, and 4) read the results. The results are interpreted by the Cepheid OS X software and are shown in the "Result Summary Page". Results from the Xpert HF test are used as an aid in the diagnosis of viral hemorrhagic fevers and should be used in conjunction with other clinical, epidemiologic and laboratory data.

The device was cleared previously in 510(k) K253653.

B Test System Components

The GeneXpert Edge X system consists of an instrument and a mobile tablet with an integrated barcode scanner and pre-loaded software for performing the test and viewing the results. The GeneXpert Edge X system automates and integrates sample extraction, nucleic acid purification and amplification, and detection of target sequences from EDTA-treated whole blood specimens by using reverse transcription (conversion of RNA templates into DNA) followed by real-time PCR.

The GeneXpert Edge X system requires the use of a single-use disposable GeneXpert cartridge that contains target-specific reagents and carries out the RT-PCR and PCR processes. The cartridges are self-contained minimizing the risk of cross-contamination between samples.

The Xpert HF test includes reagents for the detection of RNA from Ebola virus (including Zaire, Sudan, Taï Forest, and Bundibugyo, not differentiated), Crimean-Congo hemorrhagic fever (CCHF) virus, Marburg virus, and Lassa virus in EDTA-treated capillary or venous whole blood specimens. The primers and probes in the Xpert HF test are designed to amplify and detect unique sequences in the genes that encode the following protein: Ebola nucleoprotein (NP), Lassa glycoprotein precursor (GPC), CCHF polymerase (L), and Marburg nucleoprotein (NP). A Sample Processing Control (SPC), a Sample Adequacy Control (SAC), and a Probe Check Control (PCC) are also included in the cartridge.

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

- The Xpert HF test is a self-contained test: PCR reagents and processes are hosted in a single use and self-contained GeneXpert cartridge.
- Direct, unprocessed specimens are used: capillary whole blood and venous whole blood.
- Specimen manipulation is non-technique-dependent: whole blood specimens are collected in EDTA anticoagulant collection tubes, transferred directly to off-board Sample Reagent vials for inactivation, mixed by inversion, and transferred into test cartridges. Pre-analytic sample handling requirements for the Xpert HF test are simple specimen and/or 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.
- Operator intervention is not required during analysis steps. When the Xpert HF test is complete, results are displayed by the GeneXpert Edge X software.
- Technical or specialized training is not required for troubleshooting or error code interpretation. If an error code is shown, simple instructions are provided to the user in the Quick Reference Instructions (QRI) and package insert. In addition, the Getting Started Guide and GeneXpert Edge X Basic User's Guide provides information regarding error handling and provides the phone number for contacting technical support. The Xpert HF Instructions for Use include 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 Edge X 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 Basic 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 Edge X 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 requires any additional interpretation or calculation.
- Results are easy to determine ("DETECTED"/"NOT DETECTED" for the Ebola, Lassa, CCHF, and Marburg analytes) and any result that calls for a retest is clearly indicated ("INSTRUMENT ERROR" or "NO RESULT – REPEAT TEST").
- Quick Reference Instructions that are written at no higher than a 7th grade level, that were shown to be appropriate for the intended users, are provided with the Xpert HF test kit. Quick Reference Instructions are also provided in the GeneXpert Edge X Getting Started Guide provided with the GeneXpert Edge X system at the time of delivery.

B Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-Safe Mechanisms

1. <u>Risk Analysis:</u>

A systematic and comprehensive risk analysis was conducted to identify potential sources of error for hazards and hazardous situations associated with the Xpert HF test performed on the GeneXpert Edge X system in a CLIA-waived environment, including:

- Operator Error / Human Factors
- Specimen Integrity and Handling
- Reagent Integrity
- Hardware, Software, and Electronic Integrity
- Stability of Calibration and Internal Controls
- Environmental Factors and
- Foreseeable Misuses

The risk analysis was performed according to ISO 14971 Medical Devices - Application of Risk Management to Medical Devices and Cepheid's internal risk management process.

Based on the results of the risk analysis, nine (9) potential sources of error were identified to be associated with the Xpert HF test performed on the GeneXpert Edge X system in a CLIA-waived environment:

- Incorrect Handling (Mixing) of Sample and Sample Reagent (SR)
- Incorrect Handling (Mixing) of External Controls and SR
- Incorrect Handling of Cartridge
- Incorrect Storage of Sample (Specimen in SR)
- Incorrect Storage of Specimen
- Incorrect Pipette/Incorrect Sample Volume Transferred
- Incorrect Assay Testing Environment
- Incorrect Handling of the GeneXpert Edge X system
- Miscellaneous – Sources of Other Potential Error

2. Fail-Safe and Failure Alert Mechanisms:

The Xpert HF test performed on the GeneXpert Edge X system is designed with numerous fail-safe and failure alert mechanisms to prevent erroneous results. **Table 1** describes the Fail-Safe and Failure Alert Mechanisms of the GeneXpert Edge X system.

Table 1. Fail-Safe and Failure Alert Mechanisms of the GeneXpert Edge X 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 Edge X 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.
Instrument Self-Test	• The GeneXpert Edge X 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 Edge X Instrument	• The GeneXpert Edge X 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 Pipets

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

Internal Controls

The GeneXpert Edge X System relies on the built-in internal controls included in each Xpert HF test cartridge to ensure the test system is performing as expected. The internal controls consisted of a Sample Processing Control (SPC), a Probe Check Control (PCC), and a Sample Adequacy Control (SAC). The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability. The SAC ensures the sample was correctly added to the cartridge.

External Controls

External controls (ECs) are not required for the end users to obtain a valid Xpert HF test result. External controls are not provided with the Xpert HF test and are available

from LGC Clinical Diagnostics. Specifically, the following external controls have been validated for use with the Xpert HF test:

- AccuPlex Viral Fever-ELCM Control Pack (P/N 0505-0410)
  - Viral Fever ELCM Positive Control
  - Viral Fever ELCM Negative Control

All external controls must be used in accordance with local, state, and federal accrediting organizations, as applicable. It is recommended that external controls be tested following the manufacturer's instruction at the frequency noted below:

- Each time a new lot of Xpert HF kits is received.
- Each time a new shipment of Xpert HF 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 before).
- When problems (storage, operator, instrument, or other) are suspected or identified.
- If otherwise required by your institution's standard Quality Control (QC) procedures.

Self-Check

The GeneXpert Edge X system has an internal function for on-going internal performance monitoring and if the data indicate that maintenance is required, the user will be instructed to contact Cepheid Technical Support, in which the company will send a support technician to the user.

The functionality of the fail-safe mechanisms built into the software of the Xpert HF test on the GeneXpert Edge X system are tested as described below in **Table 2**. For any error messages that may appear on-screen, the user is instructed to follow the on-screen instructions.

Table 2. Fail-Safe Mechanisms for Xpert Hemorrhagic Fever with the GeneXpert Edge X Instrument

Item	Operator Action/Condition	Expected Results
1	Ambient temperature of the module is above 45°C.	Result Summary page displays "INSTRUMENT ERROR" Error 1001: The actual temperature [XX°C] has drifted too far away from the setpoint of 46°C. The QRI contains recommended environmental operating conditions of 15-30°C.
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?"

		Result Summary page displays test result 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.	Error Message: “Cartridge has already been used. Cartridges can only be used once. Select a new cartridge and try again.” Testing does not proceed.
4	Operator turned off (unplugged) the instrument from an electrical outlet/battery power before the test was completed followed by restoration of power to the instrument.	First Message: Module Communication Lost Module(s): Module [X] lost communication while test was running. Attempting recovery. Code 2123. Second Message: Module Communication Restored Communication was restored for module(s): Module [X]. Code 2124. The QRI contains a warning to not power off the instrument while a test is in progress as this will stop the test.
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: Module Communication Lost Module(s): Module [X] lost communication while test was running. Attempting recovery. Code 2123. Second Error Message: “Cartridge has already been used. Cartridges can only be used once. Select a new cartridge and try again.” Testing does not proceed.
6	Operator powers off the tablet before the test is complete and powers the tablet back on while the test is running.	Result Summary page displays “NO RESULT-REPEAT TEST”. The QRI contains a warning to not power off the tablet while a test is in progress as this will stop the test.
7	Ethernet cable connecting the tablet to the GeneXpert Edge X system was disconnected before completion of the	First Message: Module Communication Lost Module(s): Module [X] lost communication while test was running. Attempting recovery. Code 2123.

	test and reconnected to the same tablet after completion of the test.	Second Message: Module Communication Restored Communication was restored for module(s): Module [X]. Code 2124. Result Summary will display test valid test results.
8	External Battery dies in the middle of testing.	Result Summary page displays "INSTRUMENT ERROR". User Interface contains a warning "To avoid losing a test: check the warning label on the moveable battery and make sure that the proper charge level is met."
9	Operator attempts to run cartridges beyond the expiration date.	Error Message: "Cartridge expired on [YYYY/MM/DD]. Please select a valid Cartridge and try again." 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. There is a note in the package insert to not use expired cartridges.
10	Cartridge reaction tube is damaged.	Testing proceeds but "NO RESULT – REPEAT TEST" is obtained. 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 or damaged.
11	Start a test using a cartridge that has already been used on the same instrument as the spent cartridge.	Error Message: "Cartridge has already been used. Cartridges can only be used once. Select a new cartridge and try again." Testing does not proceed. Package insert and QRI contain a warning to not reuse 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. Package insert and QRI contain a warning to not reuse cartridges.

3. Flex Studies:

Based on the potential sources of error identified in the risk analysis, flex studies were performed with the Xpert HF test on the GeneXpert Edge X system. The flex studies were designed to evaluate the insensitivity of the test system to environmental and usage variations under conditions of stress that may reasonably be expected to occur with untrained operators in intended use CLIA-Waived settings. The test conditions were also designed to verify and/or validate the effectiveness of control measures at operational limits.

Fresh K2-EDTA whole blood (WB) matrix was used to prepare both positive and negative samples used in the studies. Negative blood samples consisted of K2-EDTA WB only. Positive blood samples consisted of K2-EDTA WB prepared with inactivated Ebola, Lassa, CCHF and Marburg viruses each at 3x the analytical limit of detection (LoD). The strain and concentration of each virus used in the flex studies are listed in **Table 3**.

Table 3. Xpert HF Target Analyte Strains and Concentrations Used in Flex Studies

Virus Strain	3x LoD (copies/mL)
Ebola Zaire Mayinga (Gamma-irradiated)	2778.3
Lassa virus, Josiah (Gamma-irradiated)	3282.9
CCHF IbAr10200 (Gamma-irradiated)	1132.2
Marburg Angola (Gamma-irradiated)	1996.8

Replicates of five positive and five negative samples were tested for each test condition as described in **Table 4**. Positive and negative control samples were also tested in the study and results from each of the flex study test conditions were compared to the control conditions. Unless otherwise specified by the test condition, 200 µL single-use exact volume transfer pipettes were used for adding the sample to the SR vial and 1.1 mL transfer pipettes were used to load the SR-treated sample into the cartridge. For CWB testing, precision pipettes were used to allow for focus on the CWB specimen stability aspect with the intention to minimize variability across different timepoints and due to limited amount of CWB available per donor.

Table 4. Potential Sources of Error Evaluated in the Flex Studies

Incorrect Handling (Mixing) of Sample and Sample Reagent (SR)
Incorrect Handling (Mixing) of External Controls and SR
Incorrect Handling of Cartridge
Incorrect Storage of Sample (Specimen in SR)
Incorrect Storage of Specimen
Incorrect Pipette/Incorrect Sample Volume Transferred
Incorrect Assay Testing Environment
Incorrect Handling of the GeneXpert Edge X system
Miscellaneous - Sources of Other Potential Error

I. Incorrect Handling (Mixing) of Sample and Sample Reagent (SR)

This study evaluated the effect of improper sample handling and mixing on the performance of the Xpert HF test. The sample handling procedure described in the QRI and package insert requires addition of 200 µL of specimen to the SR vial using a fixed volume transfer pipette, then inversion of the SR vial 15 times after the sample has been added. The sample-SR mixture is incubated for 10 minutes and then the SR vial is inverted three times. A 1.1 mL

transfer pipette is used to transfer 1 mL of sample-SR mixture to the test cartridge. Twelve different improper mixing techniques were tested to evaluate the risk of erroneous results.

a. Sample was not mixed and not incubated at all before adding to the cartridge. All samples generated expected results.

b. Sample was inverted once before incubation and once before adding to the cartridge. All samples generated expected results.

c. Sample was inverted 3 times before incubation and 3 times before adding to the cartridge. All samples generated expected results.

d. Sample was inverted 3 times before incubation and 15 times before adding to the cartridge. All samples generated expected results.

e. Sample was inverted 13 times before incubation and 15 times before adding to the cartridge. All samples generated expected results.

f. Sample was mixed by vortex at maximum speed for 5 seconds before incubation and vortex at maximum speed for 5 seconds before adding to the cartridge. All samples generated expected results.

g. Sample was inverted 15 times before incubation and not mixed before adding to the cartridge. All samples generated expected results.

h. Sample was not mixed before incubation and mixed 3 times before adding to the cartridge. All negative samples generated expected results. Four of 5 positive samples generated expected results, and 1 of 5 positive samples reported “Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED” as the test result.

i. 200 µL of sample was added directly into the cartridge by 200 µL exact transfer pipette (sample was not added to SR vial). All negative samples generated expected results. All positive samples reported “NO RESULT- REPEAT TEST” test results. Probe check failures were also reported for all positive samples.

j. 1 mL of WB specimen was added directly into the cartridge by 1.1mL transfer pipette (sample was not added to SR vial). All negative samples and 4 of 5 positive samples reported “INSTRUMENT ERROR” test results, and 1 of 5 positive samples reported “NO RESULT – REPEAT TEST.”

k. Sample was prepared with Lithium Heparin VWB instead of K2-EDTA VWB. All samples generated expected results.

l. Sample was prepared with plasma from K2-EDTA VWB instead of with K2-EDTA VWB. All samples generated expected results.

To mitigate the risk of incorrect handling of samples prior to addition to the test cartridge, sample preparation instructions are provided in the QRI and package insert.

II. Incorrect Handling (Mixing) of External Controls (EC) and SR

This study evaluated the effect of improper handling and mixing of EC on the performance of the Xpert HF test. The EC testing procedure described in the QRI and package insert requires addition of 200 µL of positive or negative EC solution to the SR vial using a fixed volume transfer pipette, then inversion of the SR vial 15 times after the sample has been added. The EC-SR mixture is incubated for 10 minutes and then the SR vial is inverted three times. A 1.1 mL transfer pipette is used to transfer 1 mL of EC-SR mixture to the test cartridge. Thirteen different improper mixing techniques were tested to evaluate the risk of erroneous results.

a. Sample was not mixed and not incubated at all before adding to the cartridge. All samples generated expected results.

b. Sample was inverted once before incubation and once before adding to the cartridge. All samples generated expected results.

c. Sample was inverted 3 times before incubation and 3 times before adding to the cartridge. All samples generated expected results.

d. Sample was inverted 3 times before incubation and 15 times before adding to the cartridge. All samples generated expected results.

e. Sample was inverted 15 times before incubation and 15 times before adding to the cartridge. All samples generated expected results.

f. Sample was mixed by vortex at maximum speed for 5 seconds before incubation and vortex at maximum speed for 5 seconds before adding to the cartridge. All samples generated expected results.

g. Sample was inverted 15 times before incubation and not mixed before adding to the cartridge. All samples generated expected results.

h. Sample was not mixed before incubation and was inverted 3 times before adding to the cartridge. All samples generated expected results.

i. 200 µL of EC solution was added into the cartridge by 200 µL exact transfer pipette (EC solution was not added to SR vial). Two of 5 negative EC samples and 2 of 5 positive EC samples reported “QC Negative: ERROR” and “QC Positive: ERROR”, respectively, and “INSTRUMENT ERROR” test results. Three of 5 negative EC samples reported “QC Negative: Fail” and “NO RESULT - REPEAT TEST” test results. Three of 5 positive EC samples reported “QC Positive: Fail” and “NO RESULT - REPEAT TEST” test results.

j. The entire EC solution was added into the cartridge by 1.1 mL transfer pipette (EC solution was not added to SR vial). Three of 5 negative EC samples and 2 of 5 positive EC samples reported “QC Negative: ERROR” and “QC Positive: ERROR”, respectively, and “INSTRUMENT ERROR” test results. Two of 5 negative EC samples reported “QC Negative: Fail” and “NO RESULT - REPEAT TEST” test

results. Three of 5 positive EC samples reported "QC Positive: Fail", of which 1 replicate reported "Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg NOT DETECTED" and 2 replicates reported "Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED" test results.

k. EC sample (after mixing with SR) was incubated at 35°C overnight. All samples generated expected results.

l. EC solution was incubated at 35°C for 1 hour before adding to SR. All samples generated expected results.

m. EC solution was made by adding 200 µL of water (incorrect volume) into the lyophilized EC vials by 200 µL exact volume transfer pipette. All samples generated expected results.

The risk of incorrect handling of ECs is mitigated by the instructions provided for running ECs in the Xpert HF QRI and package insert.

III. Incorrect Handling of Cartridge

The following twenty-five conditions were tested to evaluate the risk of erroneous results due to incorrect handling of the cartridge.

a. Cartridge was dropped before adding sample. All samples generated expected results.

b. Cartridge was knocked over after adding the sample. All negative samples of 4 of 5 positive samples generated expected results. One of 5 positive samples reported "Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED" test result. The QRI and package insert for the Xpert HF test state "do not tilt cartridge after adding the sample" for this condition.

c. Cartridge was shaken (15 times) before adding sample. All samples generated expected results.

d. Cartridge was shaken after adding sample. After the cartridge was shaken 2-3 times, the sample leaked outside of the cartridge. The QRI and package insert for the Xpert HF test state "do not shake cartridge after adding the sample" for this condition.

e. Stored Xpert HF kit (cartridge, sample reagent, transfer pipettes) at -15°C for 3 days. Thawed cartridge at room temperature for 30 mins and tested the kits. All samples generated expected results.

f. Stored Xpert HF kit (cartridge, sample reagent, transfer pipettes) at -15°C for 3 days. After taking out the cartridge, immediately prepared the sample and tested (did not thaw the cartridge at room temperature). All samples generated expected results.

g. Stored Xpert HF kit (cartridge, sample reagent, transfer pipettes) at 50°C for 1 month. All samples generated expected results.

h. Stored Xpert HF kit (cartridge, sample reagent, transfer pipettes) in high Relative Humidity (90%) at 35°C for 1 week. All samples generated expected results.

i. Added sample in the cartridge and waited for 1 hr before starting test. All samples generated expected results.

j. Added sample in the cartridge and waited for 2.5 hrs before starting test. All samples generated expected results.

k. Added sample in the cartridge and waited for 4 hrs before starting test. All samples generated expected results.

l. Placed Sample ID or Patient ID label on top of the cartridge blocking the plunger. All samples generated expected results.

m. Did not fully close the cartridge lid when loaded the cartridge to the GeneXpert Edge X. All samples generated expected results.

n. Cartridge with a broken reaction tube was tested. All samples reported, “NO RESULT – REPEAT TEST.” The QRI and package insert state “do not use a cartridge that has a damaged reaction tube” for this condition.

o. Loaded Assay Definition Files for the Xpert Tropical Fever test onto the Edge X system and tested an Xpert Tropical Fever cartridge (incorrect cartridge) prepared with positive and negative VWB Xpert HF test samples. All samples reported, “Zika NOT DETECTED; Dengue NOT DETECTED; Chikungunya NOT DETECTED; Plasmodium species NOT DETECTED; Leptospira species NOT DETECTED” test results. The Xpert Tropical Fever test results would alert the end user that a wrong test (ADF and cartridge) was used.

p. Loaded Assay Definition Files for the Xpert Tropical Fever test onto the Edge X system and tested an Xpert Tropical Fever cartridge (incorrect cartridge) prepared with positive and negative external control samples for the Xpert HF test. All negative external control samples reported “QC Negative: Pass” and “Zika NOT DETECTED; Dengue NOT DETECTED; Chikungunya NOT DETECTED; Plasmodium species NOT DETECTED; Leptospira species NOT DETECTED” test results. Although the QC result was marked as "pass", a user would be able to identify that the wrong test was used for the QC. All positive external control samples reported “QC Positive: Fail” and “Zika NOT DETECTED; Dengue NOT DETECTED; Chikungunya NOT DETECTED; Plasmodium species NOT DETECTED; Leptospira species NOT DETECTED” test results. The Xpert Tropical Fever test results would alert the end user that a wrong test (ADF and cartridge) was used. The Xpert HF package insert also states “do not substitute Xpert Hemorrhagic Fever test regents with other reagents.”

q. Loaded Assay Definition Files for the Xpert TAP test onto the Edge X system and tested an Xpert TAP cartridge (incorrect cartridge) prepared with positive and negative VWB samples or positive and negative EC samples for the Xpert HF test. All negative VWB samples and negative EC samples reported “NO RESULT - REPEAT TEST” test results. All positive VWB samples and positive EC samples also reported “NO RESULT - REPEAT TEST.” The results screen showed “QC Negative: Error”, TAP Panel RUO v6 as the assay definition file name, and Error information which would alert the user the wrong test (assay definition file and cartridge) was used. Additionally, the Xpert HF package insert states “do not substitute Xpert Hemorrhagic Fever test reagents with other reagents.

r. Added 200 µL of positive or negative VWB sample to the Xpert Tropical Fever SR (Incorrect SR) and tested using Xpert HF cartridge. All negative samples reported “Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg NOT DETECTED” test results. All positive samples reported “Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED” test results. For this user error, the package insert for Xpert HF test states” Do not substitute Xpert Hemorrhagic Fever test reagents with other reagents”.

s. Added 200 µL of positive or negative EC to Xpert Tropical Fever SR (Incorrect SR) and tested using Xpert HF cartridge. All negative external control samples reported “QC Negative: Pass” and “Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg NOT DETECTED” test results. All positive external control samples reported “QC Positive: Pass” and “Ebola DETECTED; Lassa DETECTED; CCHF DETECTED; Marburg DETECTED” test results. For this user error, the package insert for Xpert HF test states “Do not substitute Xpert Hemorrhagic Fever test reagents with other reagents”.

t. Added 200 µL of positive or negative VWB to Xpert TAP SR (Incorrect SR) and tested using Xpert HF cartridge. All negative samples reported “Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg NOT DETECTED” test results. All positive samples reported “Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED” test results. For this user error, the package insert for Xpert HF test states “Do not substitute Xpert Hemorrhagic Fever test reagents with other reagents”.

u. Added 200 µL of positive or negative EC to Xpert TAP SR (Incorrect SR) and tested using Xpert HF cartridge. All negative external control samples reported “QC Negative: Pass” and “Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg NOT DETECTED” test results. All positive external control samples reported “QC Positive: Pass” and “Ebola DETECTED; Lassa DETECTED; CCHF DETECTED; Marburg DETECTED” test results.

v. Scanning an Xpert HF test cartridge on a GeneXpert Edge X system without the Xpert Hemorrhagic Fever assay definition file results in an error message informing the user that no test is found in the tablet and the user needs to import the corresponding assay definition file.

w. Running a spent cartridge (a cartridge that has already been used) on the same GeneXpert Edge X instrument results in an error message which appears after the cartridge barcode is scanned informing the user that the cartridge has been used.

x. Running a spent cartridge (a cartridge that has already been used) on a different GeneXpert Edge X instrument. All positive and negative samples (samples added to each cartridge prior to initial testing) reported "NO RESULT - REPEAT TEST". The QRI and package insert state "Do not reuse processed cartridges".

y. Tested 10 unopened cartridges (sample port was never opened). Nine of 10 cartridges returned "NO RESULT - REPEAT TEST". One cartridge reported "Ebola DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg NOT DETECTED" as the test result. Additional testing of 28 unopened cartridges tested on GeneXpert Edge X instruments reported "NO RESULT-REPEAT TEST" results. The false positive could not be replicated and the cause is unknown. Both the package insert and QRI instruct the user to open the cartridge lid and add the sample into the sample chamber.

A total of 25 conditions for incorrect handing of cartridges were tested. The conditions which did not provide the expected results are stated as warnings and precautions in the Xpert HF package insert and QRI.

IV. Incorrect Storage of Sample (positive or negative VWB in SR)

This study evaluated the risk of erroneous Xpert HF test results due to incorrect storage of VWB in SR. Testing was conducted with samples stored outside the specified temperature conditions set in the labeling and for a longer than recommended storage time (VWB and CWB in SR: 15–30 °C for up to 28 hours, 2–8 °C up to 144 hours, or 31-35°C for up to 24 hours). Two different conditions were evaluated.

a. Following 10-minute incubation, VWB in SR sample was stored at 40°C for 8 hours. All samples generated expected results.

b. Following 10-minute incubation, VWB in SR sample was stored at -15.5°C for 73 hours. All samples generated expected results.

The two conditions for the incorrect storage of VWB samples in SR provided correct results. Incorrect storage conditions of VWB samples tested in this study did not result in non-determinate or erroneous results. Associated risks with this condition are mitigated by providing storage instructions in the Xpert HF package insert.

V. Incorrect Storage of Specimen (positive or negative VWB or CWB)

The potential for erroneous Xpert HF test results to be generated by testing VWB or CWB specimens that were stored incorrectly was assessed. Testing was conducted with specimens stored outside the specified temperature conditions set in the labeling and for a longer than recommended storage time (VWB: 15–30 °C for up to 24 hours, 2–8 °C for

up to 120 hours, or at 31-35°C for 24 hours, CWB: CWB in Microtainer tube should be transferred immediately to the SR). Four conditions were evaluated.

a. Positive and negative VWB specimens stored at 40°C for 8 hrs prior to testing. All samples generated expected results.

b. Positive and negative VWB specimens stored at -15.5°C for 73 hrs prior to testing. All samples generated expected results.

c. Incubated positive and negative CWB specimens in collection device (i.e. Microtainer) at 35°C for 30 min. All samples generated expected results.

d. Incubated positive and negative CWB specimens in collection device (i.e. Microtainer) at 35°C for 1 hr. All samples generated expected results.

All 4 conditions for the incorrect storage of CWB and VWB samples provided correct results. Incorrect storage conditions of specimens tested in this study did not result in invalid or erroneous results. Potential invalid or erroneous results are mitigated by providing storage instructions in the Xpert HF package insert.

VI. Incorrect Pipette/Incorrect Sample Volume Transferred

This study evaluated the risk of erroneous Xpert HF test results due to incorrect sample transfer. The sample handling procedure described in the QRI and package insert requires addition of 200 µL of specimen to the SR vial using a fixed volume transfer pipette, then inversion of the SR vial 15 times after the sample has been added. The sample-SR mixture is incubated for 10 minutes and then the SR vial is inverted three times. A 1.1 mL transfer pipette is used to transfer 1 mL of sample-SR mixture to the test cartridge. Eleven different conditions were evaluated.

a. Nothing was added to the SR prior to transferring 1 mL to the cartridge added to the cartridges. All samples generated "NO RESULT – REPEAT TEST" results. Both the package insert and QRI instruct the user to open the cartridge lid and add the sample into the sample chamber.

b. The cartridge lid was opened and closed without adding anything to the cartridge. All samples generated "NO RESULT – REPEAT TEST" results. Both the package insert and QRI instruct the user to open the cartridge lid and add the sample into the sample chamber.

c. Added 50 µL of negative or positive VWB to SR by precision pipette. All samples generated expected results.

d. Added 100 µL of negative or positive VWB to SR by precision pipette. All samples generated expected results.

e. Added 400 µL of negative or positive VWB to SR by exact transfer pipette (2 x 200 µL additions). All samples generated expected results.

f. 1 mL of negative or positive VWB was added to the SR by 1.1 mL transfer pipette. All negative samples and 4 of 5 positive samples generated expected results. One of 5 positive samples reported "Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED" test results.

g. 1 mL of the negative or positive VWB was added to the SR by 1.1 mL transfer pipette and 200 µL of sample was added to the cartridge by exact volume transfer pipette. Three of 5 negative VWB samples generated expected results and 2 of 5 replicates of the negative VWB sample reported "INSTRUMENT ERROR" test results. One of 5 positive VWB samples generated the expected result. Two of 5 positive VWB samples reported "Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED" test results, and 2 of 5 replicates of the positive VWB sample reported "INSTRUMENT ERROR" test results.

h. 200 µL of sample was added to the cartridge by exact volume transfer pipette. All negative VWB samples and 4 of 5 positive VWB samples generated expected results. One of 5 positive VWB samples reported "Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg DETECTED" test results.

i. 500 µL of sample was added to the cartridge by precision pipette. Four of 5 negative VWB samples generated expected results and 1 of 5 negative VWB samples reported an "INSTRUMENT ERROR" test result. Three of 5 positive VWB samples generated expected results, 1 of 5 samples reported a "Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED" test result, and 1 of 5 samples reported an "INSTRUMENT ERROR" test result.

j. Transferred 800 µL of negative or positive sample to the cartridge by precision pipette. All samples generated expected results.

k. Transferred 2 mL of negative or positive sample to the cartridge by transfer pipette (two 1 mL sample draws). All samples generated expected results.

A total of 11 conditions for transferring an incorrect volume of sample were tested. The conditions which did not provide correct results are mitigated by providing detailed instructions on pipetting and preparing the specimen in the Xpert HF package insert and QRI.

VII. Incorrect Assay Testing Environment

The potential for erroneous Xpert HF results to be generated by the combination of extremely high/low temperature and relative humidity (RH) conditions was evaluated in this study using an environmental chamber to create a specific condition. Nineteen different conditions were evaluated.

a. The GeneXpert Edge X System was incubated at low temperature (10°C) + ambient humidity (50% RH) for 1 hr. All positive and negative samples generated expected results.

b. The GeneXpert Edge X System was incubated at low temperature (15°C) + ambient humidity (50% RH) for 1 hr. All positive and negative samples generated expected results.

c. The GeneXpert Edge X System was incubated at high temperature (30°C) + ambient humidity (50% RH) for 1 hr. All positive and negative samples generated expected results.

d. The GeneXpert Edge X System was incubated at high temperature (40°C) + ambient humidity (50% RH) for 1 hr. All positive and negative samples generated expected results.

e. The GeneXpert Edge X System was incubated at ambient temperature (23°C) + high humidity (95% RH) for 1 hr. All positive and negative samples generated expected results.

f. The GeneXpert Edge X System was incubated at ambient temperature (25°C) + low humidity (20% RH) for 1 hr. All positive and negative samples generated expected results.

g. The GeneXpert Edge X System was incubated at ambient temperature (25°C) + low humidity (15.5% RH) for 1 hr. All positive and negative samples generated expected results.

h. The GeneXpert Edge X System was incubated at low temperature (10°C) + low humidity (22.9% RH) for 1 hr. All positive and negative samples generated expected results.

i. The GeneXpert Edge X System was incubated at low temperature (10°C) + high humidity (95% RH) for 1 hr. All positive and negative samples generated expected results.

j. The GeneXpert Edge X System was incubated at high temperature (45°C) + low humidity (10% RH) for 1 hr. Four of 5 negative samples reported the expected results. One of 5 negative samples reported an “INSTRUMENT ERROR” test result. One of 5 positive samples reported “Ebola NOT DETECTED; Lassa DETECTED; CCHF DETECTED; Marburg DETECTED” test results, 1 of 5 positive samples reported “Ebola NOT DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg DETECTED” test results, and 3 of 5 positive samples reported “INSTRUMENT ERROR” test results.

k. The GeneXpert Edge X System and cartridge containing the sample were incubated in conditions designed to replicate Tropical Rainforest conditions,

(≥33°C + ≥90% RH) for 1 hr. All positive and negative samples generated expected results.

l. The GeneXpert Edge X System and cartridge containing the sample were incubated in conditions designed to replicate Hot Desert conditions, (≥40°C + ≤16% RH) for 1 hr. All negative samples and 3 of 5 positive samples generated expected results. One of 5 positive samples reported "Ebola NOT DETECTED; Lassa DETECTED; CCHF DETECTED; Marburg DETECTED", and 1 of 5 positive samples reported "NO RESULT – REPEAT TEST".

   Additional testing was conducted in which both the cartridge and the sample vial were incubated separately under Hot Desert condition for 1 hour. After incubation, the sample was added to the cartridge, and the assay was run using the GeneXpert Edge X system which was incubated under Hot Desert conditions. All positive and negative samples generated expected results.

m. The GeneXpert Edge X System and cartridge containing the sample were incubated in conditions designed to replicate Polar Tundra conditions, (≤10°C + ≥75% RH) for 1 hr. All positive and negative samples generated expected results.

n. The GeneXpert Edge X System (running on external battery power (<50% charge at the start of the test)) and cartridge containing the sample were incubated at room temperature for 1 hr. Testing was conducted in rounds until the external batter power was drained. The first round of testing evaluated 5 negative VWB samples, all reported expected results. The second and third rounds of testing evaluated 5 positive VWB samples each. All positive VWB samples reported expected results during the second round of testing. Four of 5 positive VWB samples reported expected results during the third round of testing and 1 of 5 positive VWB samples reported "INSTRUMENT ERROR" due to the battery dying in the middle of the run.

o. The GeneXpert Edge X System (running on external battery power (<50% charge at the start of the test)) and cartridge containing the sample were incubated in Tropical Rainforest conditions (33°C + 90% RH). A condensation error during the first round of testing caused all VWB samples to report "INSTRUMENT ERROR". The same error did not occur during the second, third, and fourth rounds of testing, Positive and negative VWB samples evaluated in the second and third rounds, respectively, all generated expected results. All negative VWB samples tested in the fourth round reported "INSTRUMENT ERROR" due to the battery dying during the run.

p. The GeneXpert Edge X System (running on external battery power (<50% charge at the start of the test)) and cartridge containing the sample were incubated in Polar Tundra conditions (10°C + 75% RH). Four of 5 negative VWB samples tested in the first round reported expected results, 1 of 5 negative VWB samples reported an "INSTRUMENT ERROR". All positive and negative samples evaluated in the second and third rounds generated expected results. A fourth round was not attempted due to no battery power remaining after the third round of testing.

q. The GeneXpert Edge X System (running on external battery power (<50% charge at the start of the test)) and cartridge containing the sample were incubated in Hot Desert conditions (40°C + 16% RH). All negative VWB samples in the first round and 4 of 5 positive VWB samples in the second round reported expected results. One of 5 positive VWB samples in the second round reported "Ebola DETECTED; Lassa DETECTED; CCHF DETECTED; Marburg NOT DETECTED". Three of 5 positive VWB samples tested in the third round generated expected results, 1 of 5 positive VWB samples reported "Ebola NOT DETECTED; Lassa DETECTED; CCHF DETECTED; Marburg DETECTED" and 1 of 5 positive VWB samples reported "Ebola DETECTED; Lassa NOT DETECTED; CCHF DETECTED; Marburg DETECTED". A fourth round was not attempted due to no battery power remaining after the third round of testing.

   Additional testing was conducted in which both the cartridge and the sample vial were incubated separately under Hot Desert condition for 1 hour. After incubation, the sample was added to the cartridge, and the assay was run using the GeneXpert Edge X system which was incubated under Hot Desert conditions. All negative VWB samples tested in the first round and all positive VWB samples tested in the second and third rounds reported expected results. A fourth round was not attempted due to no battery power remaining after the third round of testing.

A total of 17 conditions were evaluated in this study. Four of the conditions were within the GeneXpert Edge X system operational parameters defined in the labeling as an operating temperature of 15-30°C and relative humidity of 20%-90%. All conditions within the operational parameters generated expected results. Results from this study support that two rounds of Xpert HF testing can be completed using the GeneXpert Edge X system powered by an external battery with 40-59% charge remaining. The test screen of the user interface provides a warning symbol to check the warning label on the external battery to ensure that the proper charge level is met prior to testing.

VIII. Incorrect Handling of the GeneXpert Edge X System

The following 11 conditions were tested to evaluate the risk of erroneous results due to incorrect handling of the GeneXpert Edge X system.

a. Unplug the AC power source before the test run is completed on the instrument, after 1 hr the AC power cable was plugged in again. All positive and negative VWB samples reported "INSTRUMENT ERROR".

b. The GeneXpert Edge X system was placed next to a large centrifuge (within 1 foot) that was running at minimum 9,500 rpm to mimic external vibration impact. All positive and negative VWB samples generated expected results.

c. The GeneXpert Edge X system was lifted while running the test and placed back on the stable surface. All positive and negative VWB samples generated expected results.

d. The GeneXpert Edge X system was tilted 15 degrees during the test. All positive and negative VWB samples generated expected results.

e. The GeneXpert Edge X system venting was blocked during the test. All positive and negative VWB samples generated expected results.

f. The GeneXpert Edge X system was inadvertently "bumped" 5 times and moved 5 ft away 3 times while the instrument was running the test. All positive and negative VWB samples generated expected results.

g. The GeneXpert Edge X system was used past its performance verification period. All positive and negative VWB samples generated expected results.

h. The GeneXpert Edge X system ethernet cable was disconnected from the tablet while running the assay and reconnected to the same tablet after completion of the test run. All positive and negative VWB samples generated expected results.

i. The GeneXpert Edge X system ethernet cable was disconnected from the tablet while running the assay and reconnected to a different tablet after completion of the test run. The second tablet did not display the test results. The original tablet required a reboot to display results. After restart all positive and negative VWB samples generated "NO RESULT – REPEAT TEST".

j. The GeneXpert Edge X system tablet was powered off before the test completed and was not powered on until after completion of the test. All positive and negative VWB samples generated "NO RESULT – REPEAT TEST".

k. The GeneXpert Edge X system tablet was powered off before the test was completed and powered on while the test was still running. All positive and negative VWB samples generated "NO RESULT – REPEAT TEST".

A total of 11 conditions for incorrect handing GeneXpert Edge X system were tested. Seven conditions provided correct results, and 4 conditions provided INSTRUMENT ERROR or NO RESULT-REPEAT TEST outcomes. The QRI states: "Do NOT power off the tablet while a test is in progress, as doing so will terminate the test."

IX. Miscellaneous – Sources of Potential Error

a. Operators attempted to conduct testing and use the instrument touchscreen with single or double layers of gloves. All users could use the touchscreen, and all tests generated expected results.
b. The effects of touching the reaction tube on the cartridge with or without gloves before running test and was evaluated. All positive and negative samples generated expected results.

c. Touching the transfer pipette to the lab bench after removal from the wrapper and before use was evaluated. All positive and negative samples generated expected results.

d. An expired cartridge was used during sample preparation. After scanning cartridge barcode, an error message appears, and test cannot be started if the cartridge was expired.

e. The effect of stopping a test before completion was evaluated. All positive and negative samples generated "NO RESULT – REPEAT TEST".

f. A test was stopped before completion of the run on the instrument and then a new test using the same cartridge was started on the same GeneXpert Edge X system. An error message appears after barcode on the cartridge has been scanned, informing the user that the cartridge has been used.

For all the conditions tested, none of the potential failure modes tested in this study produced unexpected results. The fail safes and warnings in the labeling are sufficient to mitigate the risks from conditions evaluated in this study.

C Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

1. User Comparison Study

a. *Study Design*

i. Xpert Hemorrhagic Fever test performance with untrained users:

To support the use of the Xpert HF test by untrained users in a CLIA-waived environment, a study was conducted in which the performance of the Xpert HF test was compared between trained users in a laboratory setting and untrained users at three sites representative of a CLIA-Waived test settings. Paired EDTA-treated VWB and EDTA-treated CWB specimens were collected at a single US site from healthy donors. Positive samples were created by adding VWB and CWB to Sample Reagent containing pseudoviral constructs carrying viral RNA for one of the four Xpert HF analytes (Ebola virus, Marburg virus, Crimean-Congo Hemorrhagic Fever [CCHF] virus, and Lassa virus). The SR vials contrived with pseudoviral constructs were contrived to a final concentration of either 1-2X Limit of Detection (LoD) or 3X LoD. It was assumed that all specimens collected at the U.S. site were negative for VHF targets since there were no documented outbreaks of VHF in the U.S. during the study period. Clinical CWB or VWB samples in SR which did not contain pseudoviral constructs were considered negative and evaluated for NPA. The study was conducted between October 2024 and March 2025.

Trained or untrained users added the VWB or CWB specimens to SR as part of the sample preparation and subsequently tested the individually prepared contrived positive or negative specimens on the Xpert HF test. Performance of the Xpert HF test was assessed relative to the expected positive and negative results of the contrived samples and non-contrived specimens.

ii. Operators

Nine untrained operators (three per CLIA-waived site) and three trained operators (all at one laboratory site) participated in the study. All untrained operators were non-laboratory healthcare personnel with no prior experience in CLIA moderate/high complexity laboratory testing, no prior training on the Xpert HF test, and diverse educational and work experience backgrounds. Information on the operators' current job title, education level, and any laboratory or relevant work experience was provided to support untrained operators were representative of untrained, naïve operators in the CLIA-waived setting. Trained operators met the qualifications to perform CLIA moderate to high complexity testing. Xpert HF performance in the hands of untrained operators was compared to performance of the trained operators.

iii. Instructions for Use

Trained users performed testing according to the Instructions for Use (IFU) but were also provided with the Quick Reference Instructions (QRI). Untrained users performed testing according to the QRI but also had access to the IFU. Untrained users were self-trained on the Xpert HF test and the GeneXpert Edge X system but were not provided with any additional training on the test or system.

iv. Subjects

A total of 725 subjects were enrolled in the study. Each subject provided either paired CWB and VWB specimens or CWB specimens only. From the VWB specimens, four samples were prepared and from CWB specimens two samples were prepared. A total of 2910 negative and contrived positive samples (1460 VWB and 1450 CWB) were prepared. Six samples (4 VWB, 2 CWB) were excluded due to ineligibility and an additional four samples were excluded due to protocol deviations resulting in 2900 samples included in the Xpert HF testing.

Inclusion Criteria:

- Documentation that the study participant provided informed consent/assent as required by the reviewing IRB. Documented receipt of Experimental Bill of Rights all participants enrolled in applicable states. In addition, minors must have provided assent or informed consent of parent(s) or legal guardian(s). Minors were defined as less than 18 years of age, unless otherwise defined by state or country requirements.
- Participant was 14 years of age or older.
- Participant was "healthy" per the study protocol definition.
- Participant was able and agreed to provide the following specimen(s) for study Purposes, CWB specimens (≥500 µL total volume) and VWB specimen (≥1 mL total volume), or CWB specimens (≥500 µL total volume)

Exclusion Criteria:

- Healthcare professional did not feel the participant was a suitable candidate for VWB and CWB collection.

- Participant was vaccinated with a Hemorrhagic Fever live virus vaccine within the past 28 days.
- Participant was previously enrolled into this protocol.
- VWB and CWB specimens were not collected according to the manufacturer's instructions.

Subject demographic information for the prospective specimens available for performance assessments is presented in **Table 5**.

Table 5. Demographic Information for Study Subjects

Study Participants (N = 724)	
Gender	
Female	474 (65.5%)
Male	250 (34.5%)
Age Group (Years)	
14-21	82 (11.3%)
22-59	538 (74.3%)
≥60	104 (14.4%)
Race	
Asian	9 (1.2%)
Black/African American	179 (24.7%)
White	535 (73.9%)
Subject Declined to Answer	1 (0.1%)
Ethnicity	
Hispanic or Latino	5 (0.7%)
Not Hispanic or Latino	718 (99.2%)
Subject Declined to Answer	1 (0.1%)

v. Performance Analyses

The Xpert HF test on the GeneXpert Edge X system was evaluated relative to the expected results for the negative and contrived positive samples. This analysis was done for each analyte in both specimen types. Positive Percent Agreement was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that the Xpert HF test had a positive result with positive contrived samples, and false negative (FN) indicates that the Xpert HF test had a negative result with positive contrived samples. Negative Percent Agreement (NPA) was calculated as 100% x (TN / (TN+ FP)). True negative (TN) indicates that the Xpert HF test result was negative for negative samples, and false positive (FP) indicates that the Xpert HF test result was positive for negative samples.

b. *Results*

The Xpert HF test performance relative to the expected results for the detection of the Crimean-Congo Hemorrhagic Fever analyte in CWB and VWB specimen types by trained and untrained users, including the PPA and NPA are presented in **Table 6**.

Table 6. Performance of Xpert HF for CCHF in CWB and VWB with Trained and Untrained Users

		Expected Results											
		Trained User						Untrained User					
		CWB			VWB			CWB			VWB		
		Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total
Xpert HF test	Pos.	91	0	91	91	0	91	91	0	91	91	0	91
	Neg.	0	360	360	0	360	360	0	360	360	0	364	364
PPA (95% CI)		100% (95.9-100.0%)			100% (95.9-100.0%)			100% (95.9-100.0%)			100% (95.9-100.0%)		
NPA (95% CI)		100% (98.9-100.0%)			100% (98.9-100.0%)			100% (98.9-100.0%)			100% (98.9-100.0%)		

Abbreviations: CI = Confidence Interval, Neg. = Negative, Pos. = Positive

The Xpert HF test performance relative to the expected results for the detection of the Ebola analyte in CWB and VWB specimen types by trained and untrained users, including the PPA and NPA are presented in **Table 7**.

Table 7. Performance of Xpert HF for Ebola in CWB and VWB with Trained and Untrained Users

		Expected Results											
		Trained User						Untrained User					
		CWB			VWB			CWB			VWB		
		Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total
Xpert HF test	Pos.	90	0	90	90	0	90	89	0	89	90	0	90
	Neg.	0	360	360	0	360	360	1[a]	360	361	0	364	364
PPA (95% CI)		100% (95.9-100.0%)			100% (95.9-100.0%)			98.9% (94.0-99.8%)			100% (95.9-100.0%)		
NPA (95% CI)		100% (98.9-100.0%)			100% (98.9-100.0%)			100% (98.9-100.0%)			100% (99.9-100.0%)		

Abbreviations: CI = Confidence Interval, Neg. = Negative, Pos. = Positive

[a.] Discrepant analysis identified clotting in the CWB sample as the probable root cause for the FN Xpert HF test result.

The Xpert HF test performance relative to the expected results for the detection of the Lassa analyte in CWB and VWB specimen types by trained and untrained users, including the PPA and NPA are presented in **Table 8**.

Table 8. Performance of Xpert HF for Lassa in CWB and VWB with Trained and Untrained Users

		Expected Results											
		Trained User						Untrained User					
		CWB			VWB			CWB			VWB		
		Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total
Xpert HF test	Pos.	90	0	90	91	0	91	90	0	90	91	0	91
	Neg.	1[a]	360	361	0	360	360	1[a]	360	361	0	364	364
PPA (95% CI)		98.9% (94.0-99.8%)			100% (95.9-100.0%)			98.9% (94.0-99.8%)			100% (95.9-100.0%)		
NPA (95% CI)		100% (98.9-100.0%)			100% (98.9-100.0%)			100% (98.9-100.0%)			100% (99.9-100.0%)		

Abbreviations: CI = Confidence Interval, Neg. = Negative, Pos. = Positive

[a.] Discrepant analysis identified clotting in the CWB sample as the probable root cause for the FN Xpert HF test result.

The Xpert HF test performance relative to the expected results for the detection of the Marburg analyte in CWB and VWB specimen types by trained and untrained users, including the PPA and NPA are presented in **Table 9**.

Table 9. Performance of Xpert HF for Marburg in CWB and VWB with Trained and Untrained Users

		Expected Results											
		Trained User						Untrained User					
		CWB			VWB			CWB			VWB		
		Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total	Pos.	Neg.	Total
Xpert HF test	Pos.	92	0	92	91	0	91	91	0	91	92	0	92
	Neg.	0	360	360	0	360	360	1[a]	360	361	0	364	364
PPA (95% CI)		100% (96.0-100.0%)			100% (95.9-100.0%)			98.9% (94.1-99.8%)			100% (96.0-100.0%)		
NPA (95% CI)		100% (98.9-100.0%)			100% (98.9-100.0%)			100% (98.9-100.0%)			100% (99.0-100.0%)		

Abbreviations: CI = Confidence Interval, Neg. = Negative, Pos. = Positive

[a] Discrepant analysis identified clotting in the CWB sample as the probable root cause for the FN Xpert HF test result.

c. *Non-determinate (ND) rate*

Of the 1448 CWB samples tested with the Xpert HF test, 11 ND results (1.5%, 11/724) were obtained by trained users and 10 ND results (1.4%, 10/724) were obtained by untrained users. Upon retesting, all samples produced a valid result and a final ND rate of 0% (0/724) for CWB samples.

Of the 1452 VWB samples tested with the Xpert HF test, 13 ND results (1.8%, 13/723) were obtained by trained users and 6 ND results (0.8%, 6/729) were obtained by untrained users. Upon retesting, 12 of the 13 VWB samples tested by trained users produced valid results and a final ND rate of 0.1% (1/723). For ND results obtained by untrained users, all 6 samples produced valid results and a final ND rate of 0% (0/729).

The results from the user comparison study results do not indicate widespread operator-specific performance issues nor a significant likelihood of erroneous results.

2. Device Performance with Analyte Concentrations Near the Cutoff

A precision and reproducibility study with untrained users was reviewed in K253653. Briefly, Xpert HF test performance was assessed using panels containing three to four target analytes each and were prepared at low positive (~1.5x LoD) or moderate positive (~3.0x LoD) concentrations in VWB and included negative VWB samples. Reproducibility of the Xpert HF test was assessed across three sites representative of CLIA Waived testing environments. The agreement with the expected final result interpretation is presented in **Table 10**.

Table 10. Summary of Reproducibility Study Results (Percent Agreement)

	Panel Member	Percent Agreement with 95% CI
Panel 1	Negative	100.0% (97.7%-100.0%) (161/161)
	Lassa Virus ~1.5x LoD	100.0% (97.7%-100.0%) (162/162)
	CCHF Virus ~1.5x LoD	99.4% (96.6%-99.9%) (160/161)
	Ebola Virus ~1.5x LoD	99.4% (96.6%-99.9%) (161/162)
Panel 2	Negative	99.4% (96.6%-99.9%) (161/162)
	Marburg Virus ~1.5x LoD	99.4% (96.6%-99.9%) (161/162)
	Ebola Virus ~3.0x LoD	99.4% (96.6%-99.9%) (161/162)
	Lassa Virus ~3.0x LoD	99.4% (96.6%-99.9%) (161/162)
Panel 3	Negative	96.3% (92.2%-98.3%) (156/162)
	Marburg Virus ~3.0x LoD	96.3% (92.2%-98.3%) (156/162)
	CCHF Virus ~3.0x LoD	100.0% (97.7%-100.0%) (161/161)

The study results demonstrated that untrained users were able to perform the Xpert HF test correctly and did not suggest a significant risk of erroneous results for samples near the LoD.

3. Operator Questionnaire

At the completion of the user comparison study, the participating untrained users completed an operator questionnaire to provide feedback on the ease of use of the GeneXpert Edge X system and performing the Xpert HF test. The questionnaire had 24 questions that evaluated the following general topics:

- Ease of setup of the GeneXpert Edge X System
- Performing the Xpert HF test
- Performing an Xpert HF retest
- Interpreting Xpert HF results

Eight of 9 untrained users completed the questionnaire; one user was no longer employed at the study site at the time the questionnaire was administered.

a) System Setup

Users rated three statements on a scale from 1 (strongly disagree) to 5 (strongly agree) related to the GeneXpert Edge X System setup. A summary of setup scores is presented in **Table 11**.

Table 11. Summary of GeneXpert Edge X System Setup Scores

Question	Mean Score	Percent of scores ≥3
It was easy to remove the GeneXpert instrument and all parts from the packaging.	4.3	100.0%
It was easy to set up the GeneXpert instrument.	4.1	87.5%
It was easy to understand and use the Getting Started Guide for assembling the Edge X system.	3.8	87.5%

b. Performing a Test

Users rated 8 statements on a scale from 1 (strongly disagree) to 5 (strongly agree) related to performing the Xpert HF test. The results are summarized in **Table 12**.

Table 12. Summary of Performing the Xpert HF Tests Scores

Question	Mean Score	Percent of scores ≥3
It was easy to understand and use the Quick Reference Instructions for performing the test and external controls	3.6	100.0%
It was easy to understand how to perform a test from the video.	3.8	100.0%
It was easy to understand the instructions to invert the tube.	4.5	100.0%
It was easy to understand the instructions on how to transfer a sample from the tube to the cartridge.	4.4	100.0%
It was easy to use the pipette to transfer the sample from the tube to the cartridge	4.8	100.0%
It was easy to understand the instructions for placing a cartridge into the instrument	4.6	100.0%
It was easy to understand the results of the tests performed.	4.8	100.0%
It was easy to understand what to do with the cartridge after the test was complete.	4.6	100.0%

c. Performing a Repeat Test

Users answered the following questions with a Yes/No answer:

- During the study, did you repeat a test?
- During the study, did you receive a "NO RESULT – REPEAT TEST"?
- During the study, did you receive an "INSTRUMENT ERROR"?

Users rated the following statements on a scale from 1 (strongly disagree) to 5 (strongly agree):

- It was easy to understand what to do next using the Quick Reference Instructions for a "NO RESULT – REPEAT TEST" result.
- It was easy to understand what to do next using the Quick Reference Instructions for an "INSTRUMENT ERROR" result.
- Overall, it was easy to perform the Xpert Hemorrhagic Fever test.

According to the responses, 87.5% of users (7/8) needed to repeat a test, 75.0% (6/8) received a "NO RESULT – REPEAT TEST" result, and 87.5% (7/8) received an "INSTRUMENT ERROR" result. The mean user score for questions related to ease of understanding next steps when NO RESULT – REPEAT TEST and INSTRUMENT ERROR and whether the Xpert HF test is easy to perform ranged from 3.6 to 4.3, the median user score was 4 and the mode user score was 4.

d. Result Interpretation by User

Users were presented with 7 different screenshots of Xpert HF test results and asked to interpret the result. A total of 100.0% (8/8) of users interpreted the result correctly. According to user responses, results for the Xpert HF test are easy to understand and interpret.

Based on the study operator responses to questions regarding ease of setting up the system, performing initial and repeat testing, and understanding test results, the Xpert HF test can be

performed easily from system setup to result interpretation by untrained users on the GeneXpert Edge X system.

D Labeling for Waived Devices

The labeling consists of:

1. Xpert Hemorrhagic Fever Package Insert (Instructions for Use)
2. Xpert Hemorrhagic Fever Test and GeneXpert Edge X System Quick Reference Instructions (QRI)
3. GeneXpert Edge X Basic User's Guide
4. GeneXpert Edge X Administrator User's Guide
5. GeneXpert Edge X Getting Started Guide
6. Xpert Hemorrhagic Fever Documentation and ADF Flyer
7. GeneXpert Edge X ADF Import Instructions
8. GeneXpert Edge X Security White Paper
9. Cepheid OS X Installation Instructions

The following elements are appropriately present:

- The test procedure within the QRI is written at 7th grade comprehension level.
- The QRI and the IFU identify the test as CLIA waived.
- The IFU contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The QRI and the IFU contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- The IFU contains a statement that any modification to the test or the manufacturer’s instructions will result in the test being classified as CLIA high complexity.
- The IFU and QRI provide instructions for conducting quality control procedures.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Benefit-Risk Considerations

The Xpert HF test detects nucleic acids from multiple biothreat agents in whole blood specimens from individuals with signs and symptoms of the suspected infections and/or who are at risk of exposure or may have been exposed to these agents. All hazards have been adequately mitigated through a combination of design features, fail-safe mechanisms, manufacturing controls, analytical verification, usability validation, detailed labeling instructions, and limiting use of the test to U.S. Department of Defense laboratories. The intended use of the test specifies use by U.S. Department of Defense laboratories having appropriate PPE and other safety precautions, including personnel trained in the safe handling of diagnostic clinical specimens potentially containing highly infectious agents. Result reporting and additional testing and confirmation procedures should be coordinated with the appropriate public health authorities. In conjunction with other mitigations, the limited use of the test to qualified users within Department of Defense laboratories adequately mitigates risks of false results and risks of exposure to users associated with testing for biothreat pathogens.

The original clinical validation of the Xpert HF test in K253653 demonstrated no false positive results and few false negative results. Results from the clinical study comparing trained and untrained user performance also demonstrated that the risk of erroneous results in the hands of untrained users is negligible. False negative results were limited to CWB samples and clotting was attributed as the probable root cause. The IFU and QRI include detailed instructions for CWB collection and labeling includes a warning that failure to follow collection instructions may result in incorrect results. Labeling states specifically that improper specimen collection or handling may cause CWB clotting which has been identified as a cause of false negative results and mitigates the risk of false negative results in CWB samples.

Overall, the benefits of using the Xpert HF test in the intended population by qualified Department of Defense users in CLIA waived settings outweigh the residual risks. When used according to the IFU, the device provides reliable results which aid in the diagnosis of diseases caused by the target analytes, with a low likelihood of erroneous results.

XII. Conclusion

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