510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K253653

B Applicant

Cepheid

C Proprietary and Established Names

Xpert Hemorrhagic Fever

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QVR	Class II	21 CFR 866.4000 - Device To Detect And Identify Biothreat Microbial Agents In Human Clinical Specimens	MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to obtain a substantial equivalence determination for the Cepheid Xpert Hemorrhagic Fever test to be run on the GeneXpert Edge X system.

B Measurand:

The assay detects and identifies nucleic acids of the following organisms: Ebola virus, Crimean-Congo hemorrhagic fever (CCHF) virus, Marburg virus, and Lassa virus.

C Type of Test:

Multiplex Nucleic Acid Amplification Test

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The Xpert Hemorrhagic Fever test, performed on the GeneXpert Edge X system, is an automated multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative in vitro detection and identification of RNA from multiple biothreat agents:

- Ebola virus (including Zaire, Sudan, Taï Forest, and Bundibugyo, not differentiated)
- Crimean-Congo hemorrhagic fever (CCHF) virus
- Marburg virus
- Lassa virus

The Xpert Hemorrhagic Fever test uses 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 Hemorrhagic Fever test is indicated as an aid in the diagnosis of viral hemorrhagic fevers and results should be used in conjunction with other clinical, epidemiologic and laboratory data in accordance with the guidelines provided by the Centers for Disease Control and Prevention (CDC) and United States Department of Defense (DoD).

The Xpert Hemorrhagic Fever test results are for the presumptive identification of Ebola, CCHF, Marburg and Lassa viruses. Definitive identification requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be necessary. Positive results do not rule out co-infection with organisms not included in the Xpert Hemorrhagic Fever test. The organism(s) detected may not be the definite cause of symptoms. Negative results do not preclude infection with these agents and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

The Xpert Hemorrhagic Fever test is for use only by United States 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. Viral hemorrhagic fevers are nationally notifiable diseases caused by biothreat agents and reporting of these diseases should be coordinated with the appropriate Department of Defense and public health authorities.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

The Xpert HF test is performed on the GeneXpert Edge X system

IV Device/System Characteristics:

A Device Description:

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 (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 includes reagents for the detection of RNA from Ebola virus (strains Zaire, Sudan, Taï Forest, and Bundibugyo, not differentiated), CCHF virus, Marburg virus, and Lassa virus in EDTA-treated capillary or venous whole blood specimens. A Sample Processing Control (SPC), a Sample Adequacy Control (SAC), and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control adequate extraction and processing of the target sequences and to monitor for the presence of inhibitors in the PCR reaction. The SAC ensures that sufficient human cells have been added in test cartridge. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

Venous and capillary whole blood specimens collected into EDTA anticoagulant tubes are added to an off-board sample reagent (SR) vial in which the sample is inactivated. The inactivated sample is then added to the Xpert HF cartridge before being loaded onto the GeneXpert Edge X instrument, automated sample processing, and real-time RT-PCR for the detection of these viral RNAs.

The Xpert HF test is designed for use with fingerstick K_2-EDTA whole blood (CWB) or EDTA-treated whole blood obtained from venous draw (VWB). The ancillary EDTA anticoagulant whole blood collection tubes verified and/or validated for use with the Xpert HF test included:

- BD Microtainer Contact-Activated Lancet (P/N 366594 or P/N 366578) (K223243)
- BD Microtainer Blood Collection Tubes (P/N 3665974) for capillary whole blood collection
- BD Vacutainer Blood Collection Tubes for venous whole blood collection (K231373)
    - P/N 367841 (2 mL)
    - P/N 366643 (10 mL)
    - P/N 367899 (6 mL)

These ancillary collection tubes allow VWB and CWB specimens from patients to be collected, preserved and transported to the laboratory prior to analysis with the Xpert HF test.

The results are interpreted from measurement of fluorescent signals by the Cepheid OS X software and are shown in the “Result Summary Page”. The Xpert HF test provides test results for Ebola, CCHF, Marburg and Lassa. If an error is encountered, “NO RESULT-REPEAT

TEST" and "INSTRUMENT ERROR" test results are also reported and the user is instructed to repeat the test. Test results are obtained in approximately 60 minutes.

B Principle of Operation:

The Xpert HF test uses reverse transcriptase to transcribe viral RNA from Ebola, Lassa, CCHF and Marburg virus into cDNA and real-time PCR for gene-specific sequence amplification. Amplified DNA is detected by fluorogenic target-specific probe hybridization followed by 5'-nuclease cleavage of the probe to release the fluorophore (TaqMan). The Xpert HF test uses lyophilized reagents in the form of freeze-dried beads, which provide enzymes, dNTPs, primers, probes and buffers to support PCR when reconstituted.

C The GeneXpert Edge X system:

The Xpert HF test is performed on the GeneXpert Edge X system, which consists of the instrument and a mobile tablet with preloaded software for performing the test and viewing the results, and an optional power unit as shown in Figure 1.

Figure 1. GeneXpert Edge X System

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 instrument has one GeneXpert module. The GeneXpert module is the basic functional operation unit common to all GeneXpert Instrument Systems. Each GeneXpert module is identical, operates independently, and 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 thermocycler for performing RT-PCR as well as detection.

The GeneXpert Edge X system uses a single-use assay-specific disposable GeneXpert cartridge that contains target-specific reagents and carries out the RT-PCR. To minimize test-to-test contamination, all fluids including amplicons and waste materials are contained within the

cartridge. Internal controls that enable the system to detect specific failure modes that could potentially result in an erroneous result are included in the cartridge and GeneXpert Edge X system. The instrument does not come into contact with any fluids within the cartridge. Each disposable cartridge is intended to test one sample. Cartridges are not re-usable.

D Instrument Description Information:

1. Instrument Name:

   The GeneXpert Edge X system running Cepheid OS X software version 1.0 or higher.

2. Specimen Identification:

   Sample ID is a unique identifier that links the sample being processed to the patient that provided the specimen. The Sample ID can be entered into the GeneXpert Edge X System either by scanning a barcode, entering the Sample ID manually using the virtual keyboard or having the system assign a random Sample ID.

3. Specimen Sampling and Handling:

   Venous and capillary whole blood specimens collected into EDTA anticoagulant tubes are added to an off-board sample reagent (SR) vial in which the sample is inactivated. The inactivated sample is then added to the Xpert HF cartridge before being loaded onto the GeneXpert Edge X instrument, which performs automated sample processing, and real-time RT-PCR for the detection of target viral RNAs.

4. Quality Control:

   Each test includes a Sample Processing Control (SPC), a Sample Adequacy Control (SAC), and a Probe Check Control (PCC).

   - **Sample Processing Control (SPC) -** The SPC is an encapsidated RNA pseudovirus in the form of a dry bead and is included in each cartridge. The SPC is mixed with the sample and verifies adequate lysis of target virus, sample processing and, detection of assay interference. The encapsidated RNA sequence is made up of recombinant fragments derived from *Yersinia enterocolitica*, *Tritrichomonas foetus* and the human genome. As such, none of the sequences have any homology to the sequence of the Ebola, Lassa, CCHF and Marburg virus genomes. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result is INVALID if all targets are reported negative and the SPC does not meet the assigned acceptance criteria.

   - **Sample Adequacy Control (SAC)** - The SAC is a housekeeping gene, TATA-binding protein gene (TBP), and ensures that sufficient human cells have been added in test cartridge.

- **Probe Check Control (PCC)** - The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The PCC passes if it meets the assigned acceptance criteria.

- **External Controls** - Following good laboratory practice, external controls, not provided in the kit, should be used in accordance with the requirements of local and state accrediting organizations, as applicable.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioFire Global Fever Special Pathogens Panel

B Predicate 510(k) Number(s):
K213362

C Comparison with Predicate(s):

Device & Predicate Device(s):	K253653	K213362
Device Trade Name	Xpert Hemorrhagic Fever	BioFire Global Fever Special Pathogens Panel
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Xpert Hemorrhagic Fever test, performed on the GeneXpert Edge X system, is an automated multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative *in vitro* detection and identification of RNA from multiple biothreat agents: • Ebola virus (including Zaire, Sudan, Taï Forest, and Bundibugyo, not differentiated) • Crimean-Congo hemorrhagic fever (CCHF) virus	The BioFire Global Fever Special Pathogens Panel is a qualitative, multiplexed, nucleic acid-based test intended for use with BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire Global Fever Special Pathogens Panel is for the simultaneous qualitative detection and identification of multiple bacterial, viral, and protozoan nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the target

	• Marburg virus • Lassa virus The Xpert Hemorrhagic Fever test uses 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 Hemorrhagic Fever test is indicated as an aid in the diagnosis of viral hemorrhagic fevers and results should be used in conjunction with other clinical, epidemiologic and laboratory data in accordance with the guidelines provided by the Centers for Disease Control and Prevention (CDC) and United States Department of Defense (DoD). The Xpert Hemorrhagic Fever test results are for the presumptive identification of Ebola, CCHF, Marburg and Lassa viruses. Definitive identification requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be necessary. Positive results do not rule out co-	pathogens described below. Pathogens identified: Chikungunya virus Dengue virus (serotypes 1, 2, 3 and 4) *Leishmania* spp. that cause visceral leishmaniasis (e.g., *L. donovani* and *L. infantum*) *Leptospira* spp. *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*) West Nile virus Pathogens presumptively identified: *Bacillus anthracis* Crimean-Congo hemorrhagic Fever virus *Ebolavirus* spp. *Francisella tularensis* Lassa virus *Marburgvirus* Yellow fever virus *Yersinia pestis* Pathogens for which the panel provides presumptive identification results require additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be necessary. Positive results do not rule out co-infections with pathogens not included on

	infection with organisms not included in the Xpert Hemorrhagic Fever test. The organism(s) DETECTED may not be the definite cause of symptoms. Negative results do not preclude infection with these agents and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. The Xpert Hemorrhagic Fever test is for use only by United States 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. Viral hemorrhagic fevers are nationally notifiable diseases caused by biothreat agents and reporting of these diseases should be coordinated with the appropriate Department of Defense and public health authorities.	the BioFire Global Fever Special Pathogens Panel. Not all pathogens that cause acute febrile illness are detected by this test, and negative results do not preclude infection with the pathogens targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Evaluation for more common causes of acute febrile illness (e.g., infections of the upper and lower respiratory tract or gastroenteritis, as well as non-infectious causes) should be considered prior to evaluation with this panel. In the United States, patient travel history, exposure risk, and consultation of the CDC Yellow Book should be considered prior to use of the BioFire Global Fever Special Pathogens Panel as some pathogens are more common in certain geographical locations. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The BioFire Global Fever Special Pathogens Panel is indicated for use in

		laboratories having appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing any of the pathogens detected by this panel. The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities. For In Vitro Diagnostic Use
Specimen Type	Venous Whole Blood and Capillary Whole Blood collected in EDTA tube	Whole blood (collected in EDTA tube
Technological Principles	Automated Nucleic Acid Extraction, Detection and Results Interpretation	Same
Single Use	Yes	Same
Assay Results	Qualitative	Same
Test Interpretation	Automated test interpretation and report generation. User cannot access raw data	Same
Time to Result	About 1 hour	Same
General Device Characteristic Differences		
Analytes Detected	Nucleic acids from:	Nucleic acids from:

	• Ebola virus (including Zaire, Sudan, Taï Forest, and Bundibugyo, not differentiated) • Crimean-Congo hemorrhagic fever (CCHF) virus • Marburg virus • Lassa virus	• *Bacillus anthracis* • Crimean-Congo hemorrhagic fever virus • *Ebolavirus* spp. • *Francisella tularensis* • Lassa virus • *Leishmania* spp. (*L. donovani* and *L. infantum*) • *Leptospira* spp. • *Marburgvirus* • Chikungunya virus • Dengue virus (serotypes 1, 2, 3 and 4) • *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax /ovale*) • West Nile virus • Yellow fever virus • *Yersinia pestis*
Internal Control	Sample Processing Control (SPC) Sample Adequacy Control (SAC) Probe Check Control (PCC)	RNA Process Control PCR2 Control Melt Analysis Control
Instrument System	Cepheid GeneXpert Edge X system	BioFire FilmArray 2.0 or BioFire FilmArray Torch systems
Software	Cepheid OS X 1.0 or higher	BIOFIRE FILMARRAY Software

VI Standards/Guidance Documents Referenced:

- ISO 14971:2019 Medical devices - Application of risk management to medical devices
- CLSI EP07-A3 Interference Testing in Clinical Chemistry; Approved Guideline – 3rd Edition
- CLSI EP37 Supplemental Tables for Interference Testing in Clinical Chemistry - 1st Edition
- CLSI EP12 Evaluation of Qualitative Binary Output Examination Performance – 3rd Edition
- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – 2nd Edition
- CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline – 2nd Edition
- IEC 61326-1:2020 Electrical equipment for measurement control and laboratory use - EMC requirements - Part 1: General requirements – 3rd Edition

- IEC 61326-2-6:2020 Electrical equipment for measurement control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment – 3rd Edition
- IEC 62304:2015 Medical device software - Software life cycle processes – Edition 1.1
- IEC 60601-1-2:2020 Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests – Edition 4.1

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The reproducibility and precision of the Xpert Hemorrhagic Fever (Xpert HF) test on the GeneXpert Edge X instrument by untrained users was evaluated in a multicenter blinded study across three sites representative of CLIA Waived testing environments using panels of contrived samples prepared in pooled EDTA-treated venous whole blood (VWB). Panels used in the study contained three to four target panel members each and were prepared at low positive (~1.5x LoD) or moderate positive (~3.0x LoD) concentrations and included negative VWB samples.

Positive samples were contrived by diluting recombinant pseudoviral constructs containing target viral RNA for each of the four analytes (Ebola, CCHF, Lassa, and Marburg) into pooled negative EDTA-treated VWB specimens. Testing was conducted to assess reproducibility over days, lots of Xpert HF cartridges, sites, and operators at each site. The percent agreement of the correct results compared to the expected results analyzed by each of the operators across site is shown in Table 1. Additionally, the combined agreement across sites for each sample (% total agreement) and the two-sided Wilson Score confidence intervals (CI) are presented in the last column.

Table 1. Summary of Reproducibility Study Results (Percent Agreement)

Panel Member	Site 1				Site 2				Site 3				% Agreement by Analyte
	Panel 1												
	Op1	Op2	Op3	Site Total	Op1	Op2	Op3	Site Total	Op1	Op2	Op3	Site Total	Total Agreement with 95% CI
Negative	100.0% (18/18)	100.0% (17/17)[a]	100.0% (18/18)	100.0% (53/53)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (97.7%-100.0%) (161/161)
Lassa Virus ~1.5x LoD	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (97.7%-100.0%) (162/162)
CCHF Virus ~1.5x LoD	100.0% (17/17)[b]	100.0% (18/18)	100.0% (18/18)	100.0% (53/53)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	94.4% (17/18)	98.1% (53/54)	99.4% (96.6%-99.9%) (160/161)
Ebola Virus ~1.5x LoD	100.0% (18/18)	100.0% (18/18)[c]	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	94.4% (17/18)	98.1% (53/54)	99.4% (96.6%-99.9%) (161/162)
	Panel 2												
	Op1	Op2	Op3	Site Total	Op1	Op2	Op3	Site Total	Op1	Op2	Op3	Site Total	Total Agreement with 95% CI

Negative	100.0% (18/18)	94.4% (17/18)	100.0% (18/18)	98.1% (53/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	99.4% (96.6%-99.9%) (161/162)
Marburg Virus ~1.5x LoD	100.0% (18/18)	94.4% (17/18)	100.0% (18/18)	98.1% (53/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	99.4% (96.6%-99.9%) (161/162)
Ebola Virus ~3.0x LoD	100.0% (18/18)	94.4% (17/18)	100.0% (18/18)	98.1% (53/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	94.4% (17/18)	100.0% (54/54)	99.4% (96.6%-99.9%) (161/162)
Lassa Virus ~3.0x LoD	100.0% (18/18)	94.4% (17/18)	100.0% (18/18)	98.1% (53/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	94.4% (17/18)	100.0% (54/54)	99.4% (96.6%-99.9%) (161/162)
	Panel 3												
	Op1	**Op2**	**Op3**	**Site Total**	**Op1**	**Op2**	**Op3**	**Site Total**	**Op1**	**Op2**	**Op3**	**Site Total**	**Total Agreement with 95% CI**
Negative	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	66.7% (12/18)	100.0% (18/18)	88.9% (48/54)	96.3% (92.2%-98.3%) (156/162)
Marburg Virus ~3.0x LoD	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (18/18)	66.7% (12/18)	100.0% (18/18)	88.9% (48/54)	96.3% (92.2%-98.3%) (156/162)
CCHF Virus ~3.0x LoD	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (17/17)[d]	100.0% (18/18)	100.0% (18/18)	100.0% (53/53)	100.0% (18/18)	100.0% (18/18)	100.0% (18/18)	100.0% (54/54)	100.0% (97.7%-100.0%) (161/161)

Abbreviation: CCHF = Crimean-Congo hemorrhagic fever, CI = Confidence Interval, Op = Operator

[a] Of the 18 samples tested, one yielded ND results on both initial and repeat testing.

[b] Of the 18 samples tested, one yielded ND results on both initial and repeat testing.

[c] One specimen, Sample ID O 1-03-06-F, from the panel member Ebola Virus (-1.5 x LoD), yielded the following test result: "Ebola DETECTED; Lassa NOT DETECTED; CCHF NOT DETECTED; Marburg DETECTED", with a Marburg Ct value of 36.6. A review of the Ct data indicated the possibility of low-level contamination of the Ebola sample with Marburg by the user at the site as the Marburg Ct value was 36.6 which is below the expected Ct level for a l.5x LoD sample. Due to the observed Marburg contamination, the Marburg result was excluded from the agreement table.

[d] Of the 18 samples tested, one yielded ND results on both initial and repeat testing.

The acceptance criteria of ≥95% positivity was met for all low positive panel members for each analyte. The CCHF moderate positive panel member met the acceptance criteria of 100% agreement with the expected positive result. For the Ebola and Lassa moderate positive panel members, 1 of 162 samples for each panel member resulted in a false negative result, respectively and did not meet the acceptance criteria of 100% positivity. Discrepant analyses determined that labeling errors were responsible for the erroneous results, and hence the results were deemed acceptable. For the Marburg moderate positive panel member, 6 of 162 samples yielded a false negative result and did not meet the acceptance criteria of 100% agreement. From Panel 1, the negative panel member demonstrated 100% (161/161) agreement; however, the negative panel member from Panel 2 (99.4%; 161/162) and Panel 3 (96.3%; 156/162) contributed to the overall failure to meet the acceptance criteria. Discrepant analysis determined that either the untrained operator failed to add the panel member sample to the sample reagent vial with the corresponding label, or mislabeled the Xpert HF cartridge with a label corresponding to another panel member for Marburg moderate positive samples and negative samples which resulted in the erroneous results. The observed percent agreement was acceptable.

No statistically significant differences in Xpert HF test performance by untrained users were observed between study sites, lots, and operators (Table 2). The results presented in the reproducibility and precision study for the Xpert HF test using untrained operators are acceptable.

Table 2. Summary of Ct Variance (by variance source)

Panel	Panel Analyte	Total number of non-zero Ct values	Mean	Variance Source											
				Between Site		Between User		Between Lot		Between Day		Within Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	Lassa Virus ~1.5x LoD	162	32.14	0.11	0.35	0.00	0.00	0.21	0.66	0.03	0.11	0.48	1.50	0.54	1.68
1	CCHF Virus ~1.5x LoD	160	32.72	0.00	0.00	0.05	0.14	0.00	0.00	0.00	0.00	0.63	1.93	0.63	1.93
1	Ebola Virus ~1.5x LoD	161	32.79	0.00	0.00	0.06	0.17	0.14	0.43	0.00	0.00	0.31	0.96	0.35	1.07
2	Marburg Virus ~1.5x LoD	161	31.31	0.00	0.00	0.00	0.00	0.36	1.14	0.00	0.00	0.65	2.08	0.74	2.38
2	Lassa Virus ~3.0x LoD	161	31.77	0.00	0.00	0.00	0.00	0.14	0.43	0.00	0.00	0.24	0.76	0.28	0.87
2	CCHF Virus ~3.0x LoD	161	31.18	0.11	0.35	0.03	0.11	0.00	0.00	0.24	0.77	0.34	1.08	0.43	1.37
3	Ebola Virus ~3.0x LoD	156	30.37	0.02	0.07	0.00	0.00	0.12	0.41	0.00	0.00	0.56	1.84	0.57	1.89
3	Marburg Virus ~3.0x LoD	161	31.59	0.00	0.00	0.14	0.46	0.07	0.22	0.00	0.00	0.35	1.12	0.39	1.23

2. Linearity:

   Not applicable for qualitative assays.

3. Analytical Specificity/Interference:

   Analytical Reactivity

   The analytical reactivity (inclusivity) of the Xpert HF test was evaluated by utilizing multiple clinically relevant strains of Ebola, Lassa, CCHF, and Marburg viruses at ~2-3x the analytical limit of detection (LoD) of the respective reference strain. Individual inclusivity strains were prepared in K_2-EDTA VWB. All strains were tested with a minimum of three replicates using the Xpert HF test. Strains that were unavailable for wet-testing were evaluated using *in silico* analysis of sequence data to predict analytical reactivity with the Xpert HF test (Table 3). If a target strain reported negative results for one replicate or more at ≤3x LoD, the strain was subsequently tested at a higher concentration. A strain was considered detected when all replicates at a test level were reported positive. Results from *in silico* analyses predict decreased Xpert HF sensitivity to Lassa Togo strain. Wet-testing results from the analytical reactivity study are presented in Table 4. Reduced sensitivity of the Xpert HF test was observed for Lassa strains Sauerwald (lineage II) and G2405 (lineage IV) with detection at 38.1x LoD and 7.4x LoD, respectively. Lower performance of the Xpert HF test against specific Lassa strains is reflected in the limitations section of the package insert. The results of the analytical reactivity study are considered acceptable.

Table 3. Strains Evaluated Using Only *in silico* Analyses for Analytical Reactivity

Virus	Strain	Lineage/Clade
Ebola	Zaire Booue 1996	NA
	Zaire Ebola virus/H.sapiens-wt/COD/2018/Tumba	
	Zaire Ebolavirus/H.sapiens-wt/COD/2020/Ituri	
	Zaire Gabon 2002	
	Zaire Kikwit 1995	
	Zaire Luebo 2007	
	Sudan Uganda 2022	
Lassa	Soromba	V
	Togo 2016/7082[a]	VI
Marburg	Ozolin	

[a] Two mismatches observed in reverse primer alignment may decrease the sensitivity of Xpert HF test toward Lassa Togo strain.

Table 4. Results from Analytical Reactivity Wet-Testing using the Xpert HF Test

Virus	Strain (Lineage/Clade)	Concentration Tested		Results	Multiples of LoD
		PFU/mL	Copies/mL		
Ebola	Zaire Makona	6	2.04E+03	EBOLA Detected	3x
	Zaire Mayinga	6	6.83E+02		3x
	Inactivated Zaire Guéckédou	409.5	2.78E+03		3x
	Sudan Boniface	30	2.72E+03		3x
	Sudan Gulu	10	3.60E+03		4x
	Bundibugyo Uganda	3	6.80E+03		3x
	Tai Forest	75	4.04E+03		3x
Lassa	Pinneo (I)	300	7.21E+03	LASSA Detected	3x
	Sauerwald (II)	4000	9.16E+04		38.1x
	Weller (III)	600	4.53E+03		3x
	G2405 (IV)	200	3.46E+03		7.4x
	Josiah (IV)	300	1.39E+03		3x
	Macenta (IV)	1	1.21E+03		2.6x
	Benin (VII)	150	8.49E+03		3.5x
Crimean-Congo Hemorrhagic Fever	DAK 8194 (I)	6	9.87E+02	CCHF Detected	3x
	UG3010 (II)	150	1.66E+04		3x
	IbAr10200 (III)	30	5.00E+02		3x
	SPU128/81/7 (III)	90	5.68E+02		3.4x
	Afg09-2990 (IV)[a]	150	9.84E+02		3x
	Chinese HY13 (IV)	12	9.52E+02		2.9x
	Pak JD-206 (IV)	6	9.17E+02		2.8x
	Drozdov (V)	1.2	5.76E+02		3x
Marburg	Angola	30	9.00E+02	MARBURG Detected	3x
	Ci67	30	8.10E+02		3x
	Musoke	30	7.32E+02		3x
	Inactivated Musoke	NA	3.99E+03		6x
	Ravn	6	1.18E+03		3x

Virus	Strain (Lineage/Clade)	Concentration Tested		Results	Multiples of LoD
		PFU/mL	Copies/mL		
	Inactivated Ravn	NA	3.99E+03		6x
	Inactivated Voege	6.93	2.00E+03		3x

[a] Four replicates instead of 3 were tested for this strain. All 4 valid replicates reported CCHF Detected.

Analytical Specificity and Cross-Reactivity

The analytical specificity of the Xpert HF test was evaluated by testing a panel of 86 off-panel microorganisms representing bacterial, viral, and protozoa based on their clinical presentations, epidemiological distributions similar to the viral hemorrhagic fever viruses, microorganisms likely to be found in human whole blood, and near-neighbors of Xpert HF viral targets. Organisms were either wet tested by the Xpert HF test using live or inactivated strains, genomic DNA, or synthetic RNA. For hard-to-obtain organisms and/or nucleic acid targets, *in silico* exclusivity analysis was used to supplement wet testing. To evaluate cross-reactivity with on-panel organisms, each on-panel organism was individually tested in the absence of the other 3 on-panel organisms. Organisms were spiked individually into K_2-EDTA VWB for testing, at a final concentration of $\geq 10^5$ units/mL (copies/mL, PFU/mL, $TCID_{50}$/mL, IU/mL, $CEID_{50}$/mL, parasites/mL, or cells/mL) for viruses and protozoa, $\geq 10^6$ units/mL (CFU/mL or copies/mL for bacteria), or the highest possible titer available and/or clinically relevant if high concentration virus/bacteria stocks were not available. If testing of the individual non-target organism yielded a positive result, retesting was performed at lower concentrations (3~10-fold less) until the highest concentration that did not yield a false positive result was identified.

A negative control consisting of VWB matrix was tested in parallel with the off-panel microorganisms. Each test or control sample was tested in replicates of three. Results of the analytical specificity and cross-reactivity studies are presented in Tables 5 and 6, respectively. All off-panel organisms except Aigai virus were correctly reported as NOT DETECTED. Aigai virus (formerly known as CCHF AP-92) was detected at 7.49E+04 copies/mL with very late Ct values but not detected at 2.50E+04 copies/mL. CCHF AP-92 (genogroup VI or Europe-2) was reclassified as a distinct virus, Aigai virus, by International Committee on Taxonomy of Viruses (ICTV) Nairoviridae Study Group in 2022. Of note, patients infected with Aigai virus still have similar but weaker symptoms than patients infected with CCHF virus. No cross-reactivity was observed for each of the four on-panel organisms (Ebola, Lassa, CCHF and Marburg inactivated viruses) tested individually at $>10^5$ copies/mL.

In silico analyses were used to evaluate the following pathogens which were unavailable for wet testing; Andes virus, Bas-Congo virus, Lloviu virus, Lymphocytic chorimeningitis virus, Batai virus, Bombali virus, Chapare virus, Dugbe virus, Guanarito virus, Hantaan virus, Ilesha virus, Junin virus, La Crosse virus, Murray Valley encephalitis virus, Ngari virus, Nipah virus, Puumala virus, Sabia virus, Seoul virus, Severe fever with thrombocytopenia syndrome virus, Tacaribe virus, Thogoto virus, and Tick-borne encephalitis virus. No cross-reactivity with these off-panel pathogens was predicted by *in silico* analysis. The results of the analytical specificity and cross-reactivity evaluations for the Xpert HF test are acceptable.

Table 5. Analytical Specificity Results from Xpert HF Evaluation of Off-Panel Microorganisms

Organism	Strain	Concentration Tested	Test Results Reported for 3/3 Replicates			
			Ebola	Lassa	CCHF	Marburg
Virus	Adenovirus[a]	1.60E+07 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Aigai virus[a] (formerly CCHF AP92)	7.49E+05 copies/mL	Not Detected	Not Detected	**Detected**	Not Detected
		7.49E+04 copies/mL	Not Detected	Not Detected	**Detected**	Not Detected
		2.50E+04 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Chikungunya virus[a]	2.80E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Cytomegalovirus (CMV)[a]	8.89E+03 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Dengue virus (DENV-1)[a]	2.80E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Dengue virus (DENV-2)[a]	1.00E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Dengue virus (DENV-3)[a]	8.90E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Dengue virus (DENV-4)[a]	2.32E+04 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Enterovirus (EV71)[a]	1.60E+06 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Epstein Barr virus (EBV) B95-8[a]	1.21E+05 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Hendra virus[b]	2.47E+07 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Hepatitis A virus[a]	2.80E+06 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Hepatitis B virus[a]	1.64E+06 IU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Hepatitis C virus[a]	6.26E+05 IU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Hepatitis E virus[c]	3.70E+06 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	HIV1[a]	1.01E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	HIV2[a]	1.01E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Influenza A virus[a]	9.60E+06 $CEID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Influenza B virus[a]	8.89E+05 $CEID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Japanese encephalitis virus[b]	1.08E+05 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Lujo virus[a]	1.56E+05 PFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Machupo virus[a]	1.77E+05 PFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Mayaro virus (Alphavirus sp.)[a]	1.60E+06 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Measles virus[a]	1.60E+04 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected

Organism	Strain	Concentration Tested	Test Results Reported for 3/3 Replicates			
			Ebola	Lassa	CCHF	Marburg
	Mobala virus Acar3080[a]	1.33E+05 PFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Mopeia virus[a]	1.34E+05 PFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Parvovirus B19[d]	4.20E+06 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Reston virus[a]	1.00E+06 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Rift Valley Fever virus MP-12[a]	1.60E+05 PFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Rubella virus[a]	5.00E+02 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Sindbis virus[b]	2.50E+07 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Spondweni virus[a]	1.02E+05 PFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	St. Louis encephalitis virus[a]	2.80E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Usutu virus[a]	1.60E+06 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Varicella-zoster virus[a]	1.00E+03 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	West Nile virus[e]	6.80E+08 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Yellow Fever vaccine strain 17D[a]	1.60E+05 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Yellow Fever virus[a]	1.26E+05 PFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Zika virus[a]	1.60E+05 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
Bacteria	*Bacillus anthracis*[f]	2.30E+08 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Borrelia burgdorferi	N/A (1:10 dilution of stock)	Not Detected	Not Detected	Not Detected	Not Detected
	Clastridium botulinum[f]	4.58E+06 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Enterococcus faecalis	3.28E+06 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Escherichia coli	3.12E+07 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Francisella tularensis	N/A (1:10 dilution of stock)	Not Detected	Not Detected	Not Detected	Not Detected
	Haemophilus influenzae	6.33E+04 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Klebsiella pneumoniae[g]	1.02E+06 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Leptospira interrogans	2.29E+06 cells/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Listeria monocytogenes	1.70E+06 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Neisseria meningitidis	1.33E+05 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected

Organism	Strain	Concentration Tested	Test Results Reported for 3/3 Replicates			
			Ebola	Lassa	CCHF	Marburg
	Pseudomonas aeruginosa	1.00E+07 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Rickettsia monacensis	9.00E+04 $TCID_{50}$/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Salmonella enterica	3.49E+07 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Staphylococcus aureus	1.00E+06 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Staphylococcus epidermidis	2.98E+07 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Streptococcus pneumoniae	1.00E+06 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Streptococcus pyogenes	1.87E+06 CFU/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Vibrio cholerae	1.01E+06 Unit/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Yersinia pestis[f]	2.30E+08 copies/mL	Not Detected	Not Detected	Not Detected	Not Detected
Protozoa	*Leishmania braziliensis*	2.05E+06 cells/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Plasmodium falciparum	1.05E+05 parasites/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Plasmodium vivax	2.70E+05 parasites/mL	Not Detected	Not Detected	Not Detected	Not Detected
	Toxoplasma gondii	1.01E+06 cells/mL	Not Detected	Not Detected	Not Detected	Not Detected

[a] Virus (live or inactivated)
[b] Genomic RNA
[c] Synthetic RNA
[d] Synthetic DNA
[e] Plasmid
[f] Genomic DNA

Table 6. Cross-Reactivity Results from Xpert HF Evaluation of On-Panel Microorganisms

Strain	Classification	Concentration Tested	Test Results Reported for 3/3 Replicates			
			Ebola	Lassa	CCHF	Marburg
Zaire Ebolavirus	*Filoviridae*	8.87E+06 cp/mL	Detected	Not Detected	Not Detected	Not Detected
Lassa virus	*Arenaviridae*	1.45E+07 cp/mL	Not Detected	Detected	Not Detected	Not Detected
CCHF virus	*Nairoviridae*	3.31E+09 cp/mL	Not Detected	Not Detected	Detected	Not Detected
Marburg virus	*Filoviridae*	3.18E+07 cp/mL	Not Detected	Not Detected	Not Detected	Detected

Competitive Interference

To evaluate competitive interference (on-panel organisms), individual AccuPlex pseudoviral constructs containing non-infectious, non-replicative, and partial recombinant Ebola, Lassa, CCHF, and Marburg genes were diluted to ~3x limit of detection (LoD) in VWB and tested on the Xpert HF test in the presence of competing virus at high concentration ($\geq 10^5$ Cp/mL). Negative control samples consisted of Ebola, Lassa, CCHF or Marburg AccuPlex pseudoviral constructs at ~3x LoD in K_2-EDTA VWB in the absence of competing virus.

Each test condition was evaluated with a minimum of 8 valid replicates. The results for the competitive interference study are presented in Tables 7, 8, 9, and 10 for Ebola, Lassa, CCHF, Marburg, respectively. No competitive inhibitory effects were observed and the results are acceptable.

Table 7. Competitive Interference Results for Xpert HF Detection of Ebola Virus

Ebola Accuplex at 3x LoD	On-Panel Competitive Microorganism	Competitive Strain High Titer	# of Correct Test Results/ # of Valid Reps Tested
3195 copies/mL	None (Control)		8/8
	Lassa	1.00E+05 copies/mL	8/8
	CCHF	1.00E+05 copies/mL	8/8
	Marburg	1.00E+05 copies/mL	8/8

Table 8. Competitive Interference Results for Xpert HF Detection of Lassa Virus

Lassa Accuplex at 3x LoD	On-Panel Competitive Microorganism	Competitive Strain High Titer	# of Correct Test Results/ # of Valid Reps Tested
5676 copies/mL	None (Control)		8/8
	Ebola	1.00E+05 copies/mL	8/8
	CCHF	1.00E+05 copies/mL	8/8
	Marburg	1.00E+05 copies/mL	8/8

Table 9. Competitive Interference Results for Xpert HF Detection of CCHF Virus

CCHF Accuplex at 3x LoD	On-Panel Competitive Microorganism	Competitive Strain High Titer	# of Correct Test Results/ # of Valid Reps Tested
609.3 copies/mL	None (Control)		8/8
	Ebola	1.00E+05 copies/mL	8/8
	Lassa	1.00E+05 copies/mL	8/8[a]
	Marburg	1.00E+05 copies/mL	8/8

[a] One of 8 replicate tests was reported as ERROR. The run was successfully repeated to obtain 8 valid replicates

Table 10. Competitive Interference Results for Xpert HF Detection of Marburg Virus

Marburg Accuplex at 3x LoD	On-Panel Competitive Microorganism	Competitive Strain High Titer	# of Correct Test Results/ # of Valid Reps Tested
1305 copies/mL	None (Control)		8/8
	Ebola	1.00E+05 copies/mL	8/8
	Lassa	1.00E+05 copies/mL	8/8
	CCHF	1.00E+05 copies/mL	8/8

Microbial Interference

To evaluate microbial interference (off-panel organisms), AccuPlex pseudoviral constructs (Ebola, Lassa, CCHF, and Marburg) co-spiked to ~3x limit of detection (LoD) in K_2-EDTA VWB were tested in the presence of each potentially interfering microorganism at high

concentration (≥10^6 CFU/mL for bacteria and ≥10^5 PFU/mL for viruses). A negative control sample consisting of Ebola, Lassa, CCHF and Marburg AccuPlex pseudoviral constructs in the absence of potentially interfering microorganism was also tested. Each test condition was evaluated with a minimum of 8 valid replicates. The results of the microbial interference evaluation are presented in Table 11. All target analytes were detected at 100% positivity in the presence or absence of potentially interfering off-panel microorganisms and the results are acceptable.

Table 11. Microbial Interference Results for Xpert HF

Xpert HF Analyte	Off-Panel Potentially Interfering Microorganism	Concentration Tested	# of Correct Test Results/ # of Valid Reps Tested	Positivity Rate (%)
Ebola	None (Control)	N/A	8/8	100
	HBV	1.00E+05 PFU/mL	8/8	100
	HIV	1.00E+05 PFU/mL	8/8	100
	WNV	1.00E+05 PFU/mL	8/8	100
	E. coli	1.00E+06 CFU/mL	8/8	100
	S. aureus	1.00E+06 CFU/mL	8/8	100
	S. pyogenes	1.00E+06 CFU/mL	8/8	100
Lassa	None (Control)	N/A	8/8	100
	HBV	1.00E+05 PFU/mL	8/8	100
	HIV	1.00E+05 PFU/mL	8/8	100
	WNV	1.00E+05 PFU/mL	8/8	100
	E. coli	1.00E+06 CFU/mL	8/8	100
	S. aureus	1.00E+06 CFU/mL	8/8	100
	S. pyogenes	1.00E+06 CFU/mL	8/8	100
CCHF	None (Control)	N/A	8/8	100
	HBV	1.00E+05 PFU/mL	8/8	100
	HIV	1.00E+05 PFU/mL	8/8	100
	WNV	1.00E+05 PFU/mL	8/8	100
	E. coli	1.00E+06 CFU/mL	8/8	100
	S. aureus	1.00E+06 CFU/mL	8/8	100
	S. pyogenes	1.00E+06 CFU/mL	8/8	100
Marburg	None (Control)	N/A	8/8	100
	HBV	1.00E+05 PFU/mL	8/8	100
	HIV	1.00E+05 PFU/mL	8/8	100
	WNV	1.00E+05 PFU/mL	8/8	100
	E. coli	1.00E+06 CFU/mL	8/8	100
	S. aureus	1.00E+06 CFU/mL	8/8	100
	S. pyogenes	1.00E+06 CFU/mL	8/8	100

Abbreviations: HBV – Hepatitis B Virus, HIV – Human Immunodeficiency Virus, WNV – West Nile Virus

Potentially Interfering Substances

Substances that may be present in whole blood or introduced during specimen collection were evaluated for the potential to interfere with accurate detection of Ebola, Lassa, CCHF and Marburg by the Xpert HF test. Table 12 presents the 35 evaluated substances and the corresponding test concentration. Positive and negative samples were prepared in venous whole blood matrix. Negative samples (n = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC) and the sample adequacy control (SAC). Positive samples (n = 8) were tested per substance with Ebola, Lassa, CCHF and Marburg AccuPlex pseudoviral constructs prepared at 3x the limit of detection (LoD) determined for each strain. Test concentrations were determined by CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry, or CLSI EP07: Interference Testing in Clinical Chemistry. If a substance was not listed in EP37 or EP07, a clinically relevant concentration was used. A substance was considered an interferent if there was a ≥10% increase in the mean Ct value relative to the control. If interference was observed, serial titration was performed to identify and record the highest concentration of substance where interference does not occur. No interference with the Xpert HF test was observed at the concentrations tested for any of the 35 potentially interfering substances and the results are acceptable.

Table 12. Potentially Interfering Substances Evaluated by the Xpert HF Test

Category	Substance	Description	Concentration Tested
Exogenous Substances	Acetaminophen	Painkiller	0.156 mg/mL
	Acetylsalicylic acid	Painkiller	0.03 mg/mL
	Artemether	Antimalarial	0.04 µg/mL
	Artesunate	Antimalarial	0.003 mg/mL
	Atovaquone	Antiprotozoal agents	0.005 mg/mL
	Azithromycin	Macrolide Antibiotics	0.0111 mg/mL
	Caffeine	Stimulant	0.108 mg/mL
	Cetirizine HCl	Antihistamines	0.00435 mg/mL
	Prednisone	Corticosteroids	0.099 µg/mL
	Dextromethorphan	Antitussives/ Cough Syrup	0.0156 µg/mL
	Doxycycline	Tetracycline Antibiotics	0.02 mg/mL
	Heparin	Anticoagulant	3.3 units/mL
	Ibuprofen	Nonsteroidal Anti-Inflammatory Drug	0.219 mg/mL
	Imipenem	Carbapenem Antibiotics	0.1 mg/mL
	Lumefantrine	Antimalarial	0.001 mg/mL
	Mefloquine	Antimalarial	0.0035 mg/mL
	Nicotine	Stimulant	0.0199 mg/mL
	Prednisolone	Corticosteroids	0.0012 mg/mL
	Proguanil	Antimalarial	0.001 mg/mL
	Quinidine	Class IA Antiarrhythmic Agent, Antimalarial	0.0105 mg/mL
	Quinine	Antimalarial	0.01 mg/mL
	Vancomycin	Glycopeptide Antibiotics	0.12 mg/mL
Endogenous Substances	Albumin	Protein in Blood	60 mg/mL
	Bilirubin, Conjugated	Metabolic Product in Blood	0.4 mg/mL
	Bilirubin, Un-conjugated	Metabolic Product in Blood	0.4 mg/mL
	Cholesterol	Lipid in Blood	4 mg/mL
	Human Genomic DNA	Nucleic Acid in Blood	1 µg/mL
	Hemoglobin	Protein in Blood	10 mg/mL
	Immunoglobulin	Protein in Blood	20 mg/mL
	RF (Rheumatoid Factor)	Protein in Blood	40 IU/mL
	Triglycerides	Lipid in Blood	15 mg/mL
Exogenous Substances Related to Specimen Collection	Alcohol	Central Nervous System Depressant	0.5% v/v
	Hand Cream	Glycerin as Active Ingredient	1 mg/mL
	Hand Sanitizer	Ethanol as Active Ingredient	1 mg/mL
	Hand Soap	Sodium Dodecyl Sulfate as Active Ingredient	0.5% v/v

4. Assay Reportable Range:

   Not applicable for a qualitative assay.

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

   Venous Whole Blood Specimen Stability in K_2-EDTA

   The specimen stability for K_2-EDTA VWB was evaluated with positive and negative samples. Positive samples consisted of Ebola, Lassa, CCHF, and Marburg inactivated viruses

prepared in VWB at 3x LoD for each virus. The negative sample consisted of pooled normal VWB. Samples were stored at 2°C, 8°C, 15°C, 30°C, and 35°C to cover refrigerated, room temperature, and elevated temperature ranges. The samples were tested at 2°C and 8°C for up to six days, at 15°C for up to five days, and at 30°C and 35°C for up to three days. Negative and positive samples were treated with off-board Sample Reagent prior to testing at each time point. Eight replicates of each sample were evaluated at each timepoint and temperature condition.

Data from specimen stability in VWB support sample storage at room temperature (15–30 °C) for up to 24 hours, refrigerated (2–8 °C) for up to 120 hours, or at 31-35°C for 24 hours prior to Xpert HF testing.

Venous Whole Blood K_2-EDTA and Capillary Whole Blood K_2-EDTA Specimen Stability in Sample Reagent

The specimen stability for venous whole blood/capillary whole blood K_2-EDTA in Sample Reagent (SR) was evaluated with positive and negative samples stored at multiple temperatures. Positive VWB or CWB sample consisted of Ebola, Lassa, CCHF, and Marburg inactivated viruses co-spiked into a background of pooled VWB K_2-EDTA or CWB K_2-EDTA, respectively at 3x the LoD for each virus and then inactivated in SR according to the instructions for use prior to testing. Negative samples consisted of non-contrived VWB K_2-EDTA or CWB K_2-EDTA inactivated in SR. Samples were stored at 2°C, 8°C, 15°C, 30°C, and 35°C to cover refrigerated, room temperature, and elevated temperature ranges. The samples were tested at 2°C and 8°C for up to eight days, at 15°C and 30°C for up to five days, and at 35°C for up to three days. Eight replicates of each sample were evaluated at each timepoint and temperature condition.

Results from VWB K_2-EDTA and CWB K_2-EDTA specimen stability in SR support sample storage at room temperature (15–30 °C) for up to 28 hours, refrigerated (2–8 °C) up to 144 hours, or at 31-35°C for up to 24 hours prior to Xpert HF testing.

Sample Reagent Virus Inactivation Study

An inactivation study was conducted to verify the Xpert HF Sample Reagent (HFSR) inactivates Ebola virus (EBOV), Lassa virus (LASV), Crimean-Congo Hemorrhagic Fever virus (CCHV), and Marburg virus (MARV) for the use with the Xpert HF test. The live EBOV, CCHV, MARV and LASV stock concentrations were determined by plaque forming assays. Undiluted viral stocks were added directly to the HFSR to evaluate the inactivation capacity of the highest virus concentration possible. Individual virus stocks were added to HFSR in a 1:100 ratio and the mixture was inverted 15 times and incubated at room temperature for 10 minutes (as directed in the instructions for use) and for 60 minutes. Following incubation the mixture was diluted 1/100 in cell culture medium to reduce potential cytopathic effects (CPE). Virucidal efficiency of the HFSR was assessed by completing three passages using Vero E6 or SW-13 cells with each passage monitored for CPE and followed by PCR to show no detectable level of virus was present. Plaque forming assays were performed if any CPE or earlier Ct was observed over three passages to verify and quantify any live virus in the medium. Table 13 elaborates the test conditions and virus inactivation procedure.

Table 13. Xpert HFSR Test Conditions and Virus Inactivation Procedure

Panel	Condition	Treatment	Incubation	Dilution
A	Negative Control	Medium + HFSR (200 µL + 2 mL)	10 min	Medium/HFSR mixture is diluted 1/100 into medium
B	Positive Control	Virus + Medium (200 µL + 2 mL)	10 min	Virus/Medium mixture was diluted 1/100 into medium
C	Test Condition	Virus + HFSR (200 µL + 2 mL)	10 min	Virus/HFSR mixture was diluted 1/100 into medium
D			60 min	Virus/HFSR mixture was diluted 1/100 into medium

Results from the HFSR virus inactivation study are shown in Table 14. The lack of 1) CPE observed in any passages and 2) viral genomic RNA in the third passage of virus samples treated with HFSR for 10 and 60 mins demonstrates that Ebola, Crimean-Congo Hemorrhagic Fever, Lassa and Marburg viruses were effectively inactivated by the Xpert HF Sample Reagent at the indicated lengths of time. The study acceptance criteria were met and the results are acceptable.

Table 14. Inactivation Activity of HFSR on EBOV, CCHFV, MARV, and LASV in Cell Culture

Virus	Treatment	Time (mins)	Passage when CPE Observed	Passage when Ct Decreased	Initial Ct (D0P1)	Final Ct (D7P3)	Sample Inactivated
EBOV	HFSR (no virus)	10	No CPE	ND	ND	ND	NA
	EBOV + MEM	10	1	1	33.17	12.56	No
	EBOV + HFSR	10	No CPE	ND	ND	ND	Yes
	EBOV + HFSR	60	No CPE	ND	ND	ND	Yes
LASV	HFSR (no virus)	10	No CPE	ND	ND	ND	NA
	LASV + MEM	10	1	1	38.26, 38.12	18.67, 19.41	No
	LASV + HFSR	10	No CPE	ND	ND	ND	Yes
	LASV + HFSR	60	No CPE	ND	ND	ND	Yes
CCHFV	HFSR (no virus)	10	No CPE	ND	ND	ND	NA
	CCHFV + DMEM	10	1	1	ND	17.4	No
	CCHFV + HFSR	10	No CPE	ND	ND	ND	Yes
	CCHFV + HFSR	60	No CPE	ND	ND	ND	Yes
MARV	HFSR (no virus)	10	No CPE	ND	ND	ND	NA
	MARV + MEM	10	1	1	33.43	12.72	No
	MARV + HFSR	10	No CPE	ND	ND	ND	Yes
	MARV + HFSR	60	No CPE	ND	ND	ND	Yes

ND: viral RNA not detected.
D0P1: Day 0 Passage 1; D7P3: Day 7 Passage 3

6. Detection Limit:

Inactivated Virus and Accuplex Pseudoviral Construct LoD in VWB and CWB

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert HF test for each target analyte. The LoD of the Xpert HF test was first estimated using serial dilutions of AccuPlex pseudoviral constructs (Ebola, Lassa, CCHF, and Marburg) and inactivated Ebola, Lassa, CCHF and Marburg viruses, including the WHO Ebola Reference Reagent, in K_2-EDTA VWB according to the Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. Each dilution level was tested with at least 20 replicates across a minimum of three days and two reagent lots. The LoD is defined as the lowest concentration for each strain at which 95% (19/20) of replicates yield a positive result. The higher of the two estimated LoD values for each pseudoviral construct and strain, as determined by Probit regression analysis, was used for LoD verification. The LoD was verified testing a minimum of 20 replicates in VWB using one lot of Xpert HF. The verified LoD in VWB was subsequently verified in CWB matrix for a subset of the strains. The Xpert HF verified LoD concentrations for each of the target analytes in VWB and CWB on the GeneXpert Edge X system are shown in Table 15.

Table 15. Verified LoD Concentrations for Accuplex Pseudoviral Constructs and Inactivated Viruses with the Xpert HF Test

Target	Virus Strain	LoD	
		VWB	CWB
Ebola	Ebola Zaire Inactivated Virus (copies/mL)	926.1	1852.2
	Ebola Zaire AccuPlex (copies/mL)	1065	2130
	WHO Ebola Reference Reagent (units/mL)	550.6	N/A
	Ebola Sudan Inactivated Virus (copies/mL)	1830.3	N/A
	Ebola Sudan AccuPlex (copies/mL)	132.3	N/A
	Ebola Tai Forest Inactivated Virus (copies/mL)	4383.2	N/A
	Ebola BDBV Inactivated Virus (copies/mL)	4420.5	N/A
Lassa	Lassa Inactivated Virus (copies/mL)	1094.3	1094.3
	Lassa AccuPlex (copies/mL)	1892	1892
CCHF	CCHF Inactivated Virus (copies/mL)	377.4	377.4
	CCHF AccuPlex (copies/mL)	203.1	406.2
Marburg	Marburg Inactivated Virus (copies/mL)	665.6	665.6
	Marburg AccuPlex (copies/mL)	435	435

Live Virus in VWB

The LoD of the Xpert HF test with live viruses was estimated by testing K_2-EDTA VWB contrived with serial dilutions of live viruses (Ebola, Lassa, CCHF and Marburg). Due to limitations associated with Biosafety Level 4 testing, the LoD was estimated using one Xpert HF reagent lot and limited numbers of replicates and one reagent lot which was discussed with the Agency prior to testing. The estimated LoD was determined as the lowest concentration in a serial 5-fold dilution with four out of four replicates reporting positive results. The estimated LoD concentrations for the live virus strains of Ebola, Lassa, CCHF and Marburg tested with Xpert HF test are shown in Table 16 and are acceptable.

Table 16. Estimated LoD of Live Viruses in VWB with the Xpert HF Test

Virus	Strain (Lineage/Clade)	Estimated LoD	
		PFU/mL	copies/mL[a]
Ebola	Zaire Makona[b]	2	6.80E+02
	Zaire Mayinga	2	2.28E+02
	Sudan Boniface[b]	10	9.07E+02
	Bundibugyo[b]	1	2.27E+03
	Tai Forest[b]	25	1.35E+03
Lassa	Pinneo (I)[b]	100	2.40E+03
	Weller (III)	200	1.51E+03
	Josiah (IV)[b]	100	4.65E+02
CCHF	DAK8194 (I)[b]	2	3.29E+02
	UG3010 (II)	50	5.55E+03
	IbAr10200 (III)	10	1.67E+02
	Afg09-2990	50	3.28E+02
	Drozdov (V)	0.4	1.92E+02
Marburg	Angola[b]	10	3.00E+02
	Ci67	10	2.70E+02
	Musoke[b]	10	2.44E+02
	Ravn	2	3.92E+02

[a] Concentration determined by qPCR
[b] Strains used in contrived clinical study

7. Assay Cut-Off:

The Xpert HF test detects Ebola, Lassa, Crimean Congo Hemorrhagic Fever (CCHF), Marburg, a Sample Adequacy Control (SAC) and a Sample Processing Control (SPC). The Xpert HF software determines whether the SPC and SAC cycle threshold (Ct) values are within the minimum and maximum valid cycles for each control, and if so are reported as PASS, and Ct values outside the minimum and maximum valid cycles are reported as FAIL. For the Ebola, Lassa, CCHF, and Marburg analytes, Ct values within the minimum and maximum valid cycles are reported as DETECTED. Ebola, Lassa, CCHF and Marburg Ct below the minimum valid cycle number are reported as INVALID, whereas Ct values above maximum valid cycles and no Ct (Ct =0) are reported as NOT DETECTED. The minimum and maximum valid cycle Ct cutoffs are hard coded values in the Xpert HF software. Results are interpreted automatically by the GeneXpert Edge X system and are clearly shown in the "Result Summary Page".

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

An analytical study was performed to determine whether the single-use, self-contained Xpert HF cartridges prevent specimen and amplicon carry-over contamination when testing high positive and negative samples in an alternating fashion on the same GeneXpert Edge X instrument. The high positive sample consisted of a mixture of Accuplex pseudoviral constructs (Ebola Zaire, Lassa, CCHF and Marburg), each seeded at 1 x 10^6 copies/mL into

K_2-EDTA VWB and then mixed with sample reagent (SR) according to the instructions for use. Negative samples consisted of non-contrived VWB mixed with SR. The study evaluated five GeneXpert Edge X instruments. One aliquot of the negative sample was run on each instrument immediately after a cleaning procedure prior to the start of testing. Testing started with one aliquot of the high positive sample, followed immediately by one aliquot of the negative sample. The testing scheme was repeated until a total of 9 negative and 8 positive samples had been run per instrument. All negative samples were correctly reported as "NOT DETECTED" by the Xpert HF test on the GeneXpert Edge X system and no carryover contamination was observed. The results of this study are acceptable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable.

2. Matrix Comparison:

   K_2-EDTA vs. K_3-EDTA venous whole blood

   The objective of this study was to assess whether different anticoagulants affect Xpert HF performance. Venous whole blood collected in K_2- and K_3-EDTA was used to prepare contrived samples using inactivated Ebola, Lassa, CCHF, and Marburg viruses at concentrations near the LoD (0.33x LoD, 2x LoD, and 4x LoD). Negative non-contrived VWB samples were also evaluated. All samples prepared at 2x and 4x LoD demonstrated 100% positivity for both K_2-EDTA and K_3-EDTA VWB. All samples prepared at 0.33x LoD met the acceptance criteria of 10-90% positivity for both K_2-EDTA and K_3-EDTA VWB. These results support the performance of the Xpert HF test is equivalent between K2-EDTA and K3-EDTA blood collection tubes under the conditions tested and are acceptable.

C Clinical Studies:

1. Clinical Performance:

   The clinical performance of the Xpert HF test was established during a multi-center study conducted at geographically distinct study sites in the United States from January 2025 to April 2025.

   The following paired specimens were prospectively collected from consented/assented individuals during blood collection:

   - (2) ≥ 250 µL K_2-EDTA CWB BD microtainers (Becton, Dickinson and Company, Franklin Lakes, NJ, US) (hereby referred to as "microtainer")
   - (2) x ≥ 2 mL K_2-EDTA VWB blood collection tubes.

   Prospective whole blood specimens were collected from subjects meeting the following eligibility criteria:

   Inclusion criteria

- Specimen was collected from a blood donor ≥ 14 years of age
- Specimen was collected from an asymptomatic blood donor, or a symptomatic blood donor with fever lasting ≤ 10 days
- Specimens include VWB (≥ 1 mL total volume) and CWB (≥ 500 µL total volume)

Exclusion criteria

- Subject does not have or did not self-report having a fever within the past two days
- Subject was unable to provide informed consent
- Subject has participated in the study within the last 30 days.

A total of 1632 specimens (816 VWB; 816 CWB) were prospectively collected from 408 subjects during the study period. All 1632 specimens were found to be eligible for inclusion. No specimens were excluded due to protocol deviations or unresolved non-determinate Xpert HF testing. Therefore, a total of 1632 specimens were included in the eligible population and final analyses. Table 17 shows demographic information for the blood donors from which specimens were collected.

Table 17. Demographic Information for Subjects in the Prospective Clinical Study

Subpopulation	Category	N (%)
Age	14-21	7/408 (1.7%)
	22-59	319/408 (78.2%)
	≥60	82/408 (20.1%)
Sex	Male	310/408 (76.0%)
	Female	98/408 (24.0%)
Race	Black or African American	246/408 (60.3%)
	White	162/408 (39.7%)
Ethnicity	Hispanic or Latino	65/408 (15.9%)
	Not Hispanic or Latino	343/408 (84.1%)
Symptoms	Febrile	318/408 (77.9%)
	Non-Febrile	90/408 (22.1%)

Due to the low prevalence and availability of the Xpert HF target analytes, the prospective clinical study was conducted using samples contrived with live representative strains of Crimean-Congo Hemorrhagic Fever (CCHF) virus, Ebola virus, Lassa Virus and Marburg virus in paired CWB and VWB specimens. For each analyte, 408 CWB and 408 VWB specimens were contrived at concentrations of 1-2x LoD and other clinically relevant concentrations. Since specimen collection was carried out at U.S. sites only, all specimens collected were assumed to be negative for viral hemorrhagic fever (VHF) and non-contrived specimens served as negative samples since there were no documented outbreaks of VHF in the U.S. at the time of the study. No comparator method was utilized since the positive percent agreement (PPA) and negative percent agreement (NPA) of the Xpert HF test was determined relative to the expected results of contrived samples and non-contrived specimens, respectively (Table 18).

Table 18. Xpert HF vs. Expected Results

	Classification Based on Expected Results

Xpert HF Test		Positive	Negative	Total
	Positive	TP	FP	TP + FP
	Negative	FN	TN	FN + TN
	Total	TP + FN	FP + TN	TP+FP+FN+TN

Abbreviations: True Positive = TP; True Negative = TN; False Positive = FP; False Negative = FN

The clinical performance of the Xpert HF test was assessed for each target analyte based on the following equations:

- PPA = TP/(TP + FN)
- NPA = TN/(FP + TN)

The exact binomial two-sided 95% confidence interval was also calculated.

Specimens were randomized as such that the analyte status of each contrived sample and negative specimen was blinded to the operators performing the testing. Xpert HF testing was performed by trained users in accordance with the instructions for use which were provided to the sites. Positive and negative external controls were tested with the Xpert HF test prior to testing of specimens on each day study specimen testing occurred. If the initial testing result was non-determinate, the test was repeated one time using the same specimen that resulted in the non-determinate result. If the repeat was also non-determinate, the result was recorded as such.

Out of the 816 eligible CWB specimens evaluated, 9 (1.1%; 9/816) specimens yielded a non-determinate result on the initial test. After retest, 0 (0.0%; 0/816) specimens yielded a non-determinate result.

Out of the 816 VWB specimens evaluated, 10 (1.2%; 10/816) specimens yielded non-determinate results on the initial test. After retest, 0 (0.0%; 0/816) specimens yielded a non-determinate result.

The PPA and NPA of the Xpert HF test are presented below for VWB and CWB in Tables 19 and 20, respectively.

Table 19. Performance of Xpert HF Test by Concentration in VWB

Analyte	Level	PPA			NPA		
		TP/(TP+FN)	PPA (%)	95% CI	TN/(FP+TN)	NPA (%)	95% CI
CCHF	**1-2x**	51/52	98.1	89.9-99.7	N/A	N/A	N/A
	1000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	4000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	5000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	10000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	Overall	101/102	99.0	94.7-99.8	408/408	100.0	99.1-100.0
Ebola	**1-2x**	53/53	100.0	93.2-100.0	N/A	N/A	N/A
	1000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	4000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	5000x	9/9	100.0	70.1-100.0	N/A	N/A	N/A

Analyte	Level	PPA			NPA		
		TP/(TP+FN)	PPA (%)	95% CI	TN/(FP+TN)	NPA (%)	95% CI
	10000x	12/12	100.0	75.8-100	N/A	N/A	N/A
	Overall	102/102	100.0	96.4-100.0	408/408	100.0	99.1-100.0
Lassa	**1-2x**	52/52	100.0	93.1-100.0	N/A	N/A	N/A
	1000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	4000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	5000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	10000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	Overall	102/102	100.0	96.4-100.0	408/408	100.0	99.1-100.0
Marburg	**1-2x**	51/52	98.1	89.9-99.7	N/A	N/A	N/A
	1000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	4000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	5000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	10000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	Overall	101/102	99.0	94.7-99.8	408/408	100.0	99.1-100.0

Abbreviations: TP, True Positive; FN: False Negative; TN, True Negative; FP, False Positive; PPA, Positive Percent Agreement; NPA, Negative Percent Agreement; CI, Confidence Interval, N/A = Not Applicable

Table 20. Performance of Xpert HF Test by Concentration in CWB

Analyte	Level	PPA			NPA		
		TP/(TP+FN)	PPA (%)	95% CI	TN/(FP+TN)	NPA (%)	95% CI
CCHF	**1-2x**	51/52	98.1	89.9-99.7	N/A	N/A	N/A
	1000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	4000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	5000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	10000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	Overall	101/102	99.0	94.7-99.8	408/408	100.0	99.1-100.0
Ebola	**1-2x**	52/53	98.1	90.1-99.7	N/A	N/A	N/A
	1000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	4000x	14/14	100.0	75.5-100.0	N/A	N/A	N/A
	5000x	9/9	100.0	70.1-100.0	N/A	N/A	N/A
	10000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	Overall	101/102	99.0	94.7-99.8	408/408	100.0	99.1-100.0
Lassa	**1-2x**	48/52	92.3	81.8-97.0	N/A	N/A	N/A
	1000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	4000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	5000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	10000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	Overall	98/102	96.1	90.3-98.5	408/408	100.0	99.1-100.0
Marburg	**1-2x**	50/52	96.2	87.0-98.9	N/A	N/A	N/A
	1000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	4000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A
	5000x	12/12	100.0	75.8-100.0	N/A	N/A	N/A

Analyte	Level	PPA			NPA		
		TP/(TP+FN)	PPA (%)	95% CI	TN/(FP+TN)	NPA (%)	95% CI
	10000x	14/14	100.0	78.5-100.0	N/A	N/A	N/A
	Overall	100/102	98.0	93.1-99.5	408/408	100.0	99.1-100.0
Abbreviations: TP, True Positive; FN: False Negative; TN, True Negative; FP, False Positive; PPA, Positive Percent Agreement; NPA, Negative Percent Agreement; CI, Confidence Interval, N/A = Not Applicable							

The PPA of the Xpert HF test stratified by strain were calculated relative to the expected results in VWB and CWB and presented in Table 21 and Table 22, respectively.

Table 21. Performance of Xpert HF Test by Strain in VWB

Analyte	Strain	PPA		
		TP/(TP+FN)	PPA (%)	95% CI
CCHF	**Afg09-2990**	51/51	100.0	93.0-100.0
	DAK8194	50/51	98.0	89.7-99.7
Ebola	**Bundibugyo Uganda**	27/27	100.0	87.5-100.0
	Sudan Boniface	27/27	100.0	87.5-100.0
	Tai Forest	21/21	100.0	84.5-100.0
	Zaire Makona	27/27	100.0	87.5-100.0
Lassa	**Josiah 1976**	51/51	100.0	93.0-100.0
	Pinneo	51/51	100.0	93.0-100.0
Marburg	**Angola**	50/51	98.0	89.7-99.7
	Musoke	51/51	100.0	93.0-100.0

Abbreviations: TP, True Positive; FN: False Negative; PPA, Positive Percent Agreement; CI, Confidence Interval

Table 22. Performance of Xpert HF Test by Strain in CWB

Analyte	Strain	PPA		
		TP/(TP+FN)	PPA (%)	95% CI
CCHF	**Afg09-2990**	51/51	100.0	93.0-100.0
	DAK8194	50/51	98.0	89.7-99.7
Ebola	**Bundibugyo Uganda**	26/27	96.3	81.7-99.3
	Sudan Boniface	27/27	100.0	87.5-100.0
	Tai Forest	21/21	100.0	84.5-100.0
	Zaire Makona	27/27	100.0	87.5-100.0
Lassa	**Josiah 1976**	47/51	92.2	81-5-96.9
	Pinneo	51/51	100.0	93.0-100.0
Marburg	**Angola**	51/52	98.0	89.7-99.7
	Musoke	51/52	98.0	89.7-99.7

Abbreviations: TP, True Positive; FN: False Negative; PPA, Positive Percent Agreement; CI, Confidence Interval

Crimean-Congo Hemorrhagic Fever Xpert HF Performance

As shown in Tables 19 and 20 the Xpert HF test demonstrated a PPA of 99.0% (95% CI: 94.7-99.8) and an NPA of 100.0% (95% CI: 99.1-100) for CCHF in CWB and VWB sample types. There was one false negative result observed in each of the respective sample types for the DAK8194 strain at the 1-2x LoD level (Tables 21 and 22). Discrepant analysis for the CWB sample suggested the sample may have clotted resulting in a false negative result. A conclusive root cause was not identified for the false negative result in the VWB sample type.

Ebola Xpert HF Performance

As shown in Tables 19 and 20 the Xpert HF test demonstrated a PPA of 99.0% (95% CI: 94.7-99.8) and an NPA of 100.0% (95% CI: 99.1-100.0) for Ebola in the CWB sample type. With the VWB sample type, the Xpert HF test demonstrated a PPA of 100.0% (95% CI: 96.4-100.0) and an NPA of 100.0% (95% CI: 99.1-100.0) for Ebola. One false negative result was observed for the Bundibugyo Uganda strain at the 1-2x LoD level (Tables 21 and 22). Discrepant analysis for the CWB sample suggested the sample may have clotted resulting in a false negative result.

Lassa Xpert HF Performance

As shown in Tables 19 and 20 the Xpert HF test demonstrated a PPA 96.1% (95% CI: 90.3%-98.5%) and an NPA of 100.0% (95% CI: 99.1-100.0) for Lassa in CWB. With the VWB sample type, the Xpert HF test demonstrated a PPA and NPA of 100.0% for Lassa. Four false negative results were observed with the Josiah 1976 strain at 1-2x LoD in the CWB sample type (Table 22). Discrepant analysis for two samples suggested clotting may have caused the false negative results. A potential cause for the false negative results for the remaining two samples was not determined.

Marburg Xpert HF Performance

As shown in Tables 19 and 20 the Xpert HF test demonstrated a PPA of 98.0% (95% CI: 93.1-99.5) and an NPA of 100.0% (95% CI: 99.1-100.0) for Marburg in the CWB sample type. For the VWB sample type, the Xpert HF test demonstrated a PPA of 99.0% (95% CI: 94.7-99.8) and NPA of 100.0% (95% CI: 99.1-100.0) for Marburg. Two false negative results were observed in the CWB sample type and one false negative result was observed in the VWB sample type. Of the three false negative results, two occurred with the Angola strain and one false negative result was observed in the Musoke strain (Tables 21 and 22). All three false negative results occurred at the 1-2x LoD level. Discrepant analysis of one of the CWB samples suggested the sample may have contained blood clots resulting in a false negative result. A root cause was not identified for the two remaining false negative results.

The results from this study met the acceptance criteria for PPA (≥ 95.0% (95% CI lower bound: > 89%)) and NPA (> 99.0% (95% CI lower bound: 98.0%)) for CCHF, Ebola, Lassa and Marburg analytes in both CWB and VWB specimen types. The acceptance criterion of < 5% non-determinate rate was met for CWB and VWB specimens. The results from the clinical study are acceptable.

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

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

None of the rare Xpert HF target analytes were detected during the prospective clinical study.

F Other Supportive Instrument Performance Characteristics Data:

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.