WHO Emergency Use Assessment and Listing for Ebola Virus IVDs
PUBLIC REPORT

Product: Xpert Ebola Assay
EUAL Number: EAE 0443-070-00

Abstract

In order to respond to the urgent need for quality-assured in vitro diagnostics in the event of Ebola Virus Disease (EVD) outbreak, WHO has established a WHO Emergency Quality Assessment Mechanism of In Vitro Diagnostics (IVDs) for EVD. It consists of review of any existing evidence of safety and performance; desktop review of selected manufacturing and quality management systems documentation and limited laboratory evaluation of the product.

Xpert Ebola Assay with product code **GXEBOLA-10** manufactured by Cepheid AB, Röntgenvägen 5, Solna, 171 54, Sweden was listed as eligible for WHO procurement on 8 May 2015. This public report was amended on 13 June 2019 to reflect the planned inclusion of the latest Instructions for Use.

Assay principle: The Xpert Ebola Assay is a rapid, automated test for qualitative detection of the Zaire strain of the Ebola virus. The assay is performed on the Cepheid GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time reverse transcription PCR. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the real-time reverse transcription PCR reagents and host the real-time reverse transcription processes. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate GeneXpert Dx Operator Manual or GeneXpert Infinity Operator Manual.

Intended use: The Xpert Ebola Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of RNA from the Ebola Zaire virus (detected in the West Africa outbreak in 2014) in EDTA venous whole blood from individuals with signs and symptoms of Ebola virus infection in conjunction with epidemiological risk factors.
Testing with the Xpert Ebola Assay should not be performed unless the individual meets clinical and epidemiological criteria for testing of suspected cases. Results are for the presumptive identification of Ebola Zaire virus. The definitive identification of Ebola Zaire virus infection requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required.
The diagnosis of Ebola Zaire virus infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of the Ebola Zaire virus. Negative results do not preclude Ebola Zaire or other Ebola virus infections and should not be used as the sole basis for patient management decisions. The level of Ebola

virus present in blood from individuals with early systemic infection is unknown. Due to the difficulty in obtaining clinical specimens positive for Ebola, the Xpert Ebola Assay was evaluated with limited numbers of contrived specimens spiked with live Ebola Zaire virus or Ebola Zaire virus RNA. The assay has not been evaluated with blood from individuals with Ebola Zaire virus infection.

Intended user: Clinical laboratory personnel who have received specific training on the use of the Xpert Ebola Assay on the GeneXpert Instrument Systems.

Limitation: Reactivity of the Cepheid Xpert Ebola Assay was not evaluated with isolates of the *Bundibugyo ebolavirus* and *Sudan ebolavirus* species, instead *in silico* analysis was performed to predict the risk of cross-reactivity of the Xpert Ebola Assay Zaire target oligonucleotides to non-Zaire Ebola viruses. As a result detection of Bundibugyo or Sudan viruses are unlikely but cannot be entirely ruled out.

There is only one configuration of the Xpert Ebola Assay kit which contains sufficient reagents to process 10 specimens or quality control samples.

Component	**Number per kit (GXEBOLA-10)**
GeneXpert Ebola Assay Cartridges with Integrated Reaction Tubes • Bead 1, Bead 2, and Bead 3 (freeze-dried) 1 of each per cartridge • Rinse Reagent 0.5 mL per cartridge • Elution Reagent 2.0 mL per cartridge • Binding Reagent	10 1 of each per cartridge 0.5mL per cartridge 2.0mL per cartridge 2.0mL per cartridge
Ebola Sample Reagent Bag (Sample Reagent) • Lysis Reagent (Guanidinium Thiocyanate)	1 10 x 2.5mL per bottle
Disposable 1 mL Transfer Pipettes	10
CD	1
Instructions for use	1

Materials Required but Not Provided

Material	**Catalogue number**	**Description**
GeneXpert Dx System or GeneXpert Infinity Systems	varies by configuration	GeneXpert Instrument, computer with proprietary GeneXpert Software Version 4.4a or higher, Xpertise 6.2 or higher, barcode scanner, and operator manual
Printer (optional)	NA	Contact Cepheid Technical support to arrange for the purchase of a recommended printer.
Disposable Swabs	# SWAB/E-50	
Vortex	NA	
Chlorine Bleach	NA	

Stability

Name	Storage temperature	Shelf-life
GeneXpert Ebola Assay Cartridges	2 – 28 °C	12 months
Ebola Sample Reagent Bag (Sample Reagent)	2 – 28 °C	12 months

Background information

Cepheid AB submitted an expression of interest for WHO emergency quality assessment of **Xpert Ebola Assay** on 20 October 2014.

1. Product dossier assessment

Cepheid AB was granted Emergency Use Authorization by the U.S Food and Drug Administration for the **Xpert Ebola Assay** in March 2015. The information submitted to FDA and the outcome of the review was considered sufficient to fulfil requirements for eligibility for procurement by WHO.

Safety and performance documentation assessment conclusion: acceptable.

2. Review of quality management documentation

To establish the eligibility for WHO procurement, Cepheid AB was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation, it was established that sufficient information was provided by Cepheid AB to fulfil the requirements described in the "Invitation to manufacturers of Ebola Virus In Vitro Diagnostics to submit an Expression of Interest (EOI) for Emergency Assessment by WHO".

Quality management documentation assessment conclusion: acceptable.

3. Laboratory evaluation

A limited analytical evaluation of the **Xpert Ebola Assay** was conducted by the Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg, Germany which is a WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research. The limit of detection (LOD) of the assay was verified and compared to the RealStar Filovirus Screen RT-PCR Kit 1.0 (altona Diagnostics GmbH) using simulated specimens generated by spiking cell culture supernatants containing infectious Ebola virus strain Makona into whole blood of a healthy donor. The evaluation of the **Xpert Ebola Assay** was performed on the GeneXpert-IV System.

The 95% limit of detection of the assay was found to be 82.0 RNA copies/reaction, 95% CI 39.7 to 3193.6 copies/reaction.

Laboratory evaluation conclusion: acceptable.

Commitment to WHO

As a requirement to listing, the manufacturer is required update the current version (Rev. C January 2019) of the instructions for use by 31 July 2019.

Scope and duration of procurement eligibility

The **Xpert Ebola Assay** with product code GXEBOLA-10 manufactured by Cepheid AB is considered to be eligible for WHO procurement. The assay may be used to test symptomatic individuals for EVD. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Cepheid AB must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Cepheid AB is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days. Furthermore, WHO will continue to monitor the performance of the assay in the field.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality and performance comes to WHO's attention during post-market surveillance activities.

Note that the CE-marked version of the product **Xpert Ebola Assay** with product codes GXEBOLA-CE-10 and GXEBOLA-CE-50 has not been listed through the EUAL Procedure for IVDs, as the instructions for use for the CE marked version of the product are not in agreement with the WHO interim guideline "Laboratory diagnosis of Ebola virus disease".[1]

[1] https://apps.who.int/iris/bitstream/handle/10665/134009/WHO_EVD_GUIDANCE_LAB_14.1_eng.pdf;jsessionid=A3DE1D81428B145F3277633657239395?sequence=1

Labels

1.0 Instrsutctions for Use

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Ebola Assay

Instructions for Use

For Use Under an Emergency Use Authorization (EUA) Only

REF **GXEBOLA-10**

Trademark, Patents and Copyright Statements

Cepheid®, the Cepheid logo, GeneXpert®, Xpert®, and Xpertise™ are trademarks of Cepheid.

Windows® is a trademark of Microsoft Corporation.

Armored RNA® is a registered trademark of Asuragen, Inc.

THE PURCHASE OF THIS PRODUCT CONVEYS TO THE BUYER THE NON-TRANSFERABLE RIGHT TO USE IT IN ACCORDANCE WITH THIS PACKAGE INSERT. NO OTHER RIGHTS ARE CONVEYED EXPRESSLY, BY IMPLICATION OR BY ESTOPPEL. FURTHERMORE, NO RIGHTS FOR RESALE ARE CONFERRED WITH THE PURCHASE OF THIS PRODUCT.

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden

Xpert® Ebola Assay

For use under the Emergency Use Authorization (EUA) only.

1 Proprietary Name

Xpert® Ebola

2 Common or Usual Name

Xpert Ebola Assay

3 Intended Use

The Xpert Ebola Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of RNA from the Ebola Zaire virus (detected in the West Africa outbreak in 2014) in EDTA venous whole blood from individuals with signs and symptoms of Ebola virus infection in conjunction with epidemiological risk factors.

Testing with the Xpert Ebola Assay should not be performed unless the individual meets clinical and epidemiological criteria for testing of suspected cases.

Results are for the presumptive identification of Ebola Zaire virus. The definitive identification of Ebola Zaire virus infection requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of Ebola Zaire virus infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of the Ebola Zaire virus.

Negative results do not preclude Ebola Zaire or other Ebola virus infections and should not be used as the sole basis for patient management decisions.

The level of Ebola virus present in blood from individuals with early systemic infection is unknown. Due to the difficulty in obtaining clinical specimens positive for Ebola, the Xpert Ebola Assay was evaluated with limited numbers of contrived specimens spiked with live Ebola Zaire virus or Ebola Zaire virus RNA. The assay has not been evaluated with blood from individuals with Ebola Zaire virus infection.

The Xpert Ebola Assay is for use only under Emergency use Authorization (EUA) in U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests, or in similarly qualified non-U.S. laboratories, by clinical laboratory personnel who have received specific training on the use of the Xpert Ebola Assay on the GeneXpert Instrument Systems.

Notification of Public Health authorities: Local, state and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Ebola Virus Disease (EVD). Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive or negative Xpert Ebola test result on the need for additional testing and appropriate transportation of specimens.

4 Summary and Explanation

Ebola virus disease (EVD) has occurred sporadically throughout West Africa for decades of outbreaks, but the current epidemic is the largest to date. As of February 15, 2015, over 23,000 individuals have been infected and over 9,000 have died as a result. EVD has now spread to six countries including Guinea, Liberia, Sierra Leone, Mali, USA and Spain. The burden on healthcare workers in endemic areas is also significant; to date, a total of 833 health-care workers (HCW) were infected, with over 50% mortality. Since the first discovery of Ebola virus in 1976, five Ebola species have been described: Zaire, Sudan, Côte d'lvoire (Tai Forest), Bundibugyo and Reston Ebola virus. Among these Ebola virus species, Zaire Ebola virus has affected the widest geographic regions and is the cause of the current outbreak.

The Xpert Ebola Assay uses reverse transcription polymerase chain reaction (RT-PCR) technology to achieve high sensitivity for the qualitative detection of Zaire Ebola virus total nucleic acids in whole blood specimen.

5 Principle of the Procedure

The Xpert Ebola Assay is a rapid, automated test for qualitative detection of the Zaire strain of the Ebola virus. The assay is performed on the Cepheid GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time reverse transcription PCR. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the real-time reverse transcription PCR reagents and host the real-time reverse transcription processes. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx Operator Manual* or *GeneXpert Infinity Operator Manual*.

The Xpert Ebola Assay includes reagents for the detection of Zaire Ebola virus total nucleic acids in specimens as well as a sample adequacy control and an internal control and to ensure adequate addition of sample, processing of the target and to monitor presence of inhibitor(s) in the RT and PCR reactions. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Ebola Assay kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

GeneXpert Ebola Assay Cartridges with Integrated Reaction Tubes	**10 per kit**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Rinse Reagent	0.5 mL per cartridge
• Elution Reagent	2.0 mL per cartridge
• Binding Reagent	2.0 mL per cartridge
Ebola Sample Reagent Bag (Sample Reagent)	**1 per kit**
• Lysis Reagent (Guanidinium Thiocyanate)	10 x 2.5 mL per bottle
Disposable 1 mL Transfer Pipettes	**10 per kit**
CD	

Note Safety Data Sheets (SDS) are available at www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and postmortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Ebola Assay cartridges and reagents at 2–28 °C.
- Do not use any reagents that have become cloudy or discolored.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert Instrument, computer with proprietary GeneXpert Software Version 4.4a or higher, Xpertise 6.2 or higher, barcode scanner, and operator manual
- Printer: If a printer is required, contact Cepheid Technical support to arrange for the purchase of a recommended printer.
- Disposable Swabs (catalog # SWAB/E-50)
- Vortex
- Chlorine Bleach

9 Warnings and Precautions

- For *in vitro* diagnostic use under Emergency Use Authorization only.
- Local, state, and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Ebola Virus Disease (EVD). Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive detection OR no detection (negative) EVD test result on the need for additional testing and appropriate transportation of specimens.
- All results should be interpreted by a trained professional in conjunction with review of the patient's clinical signs and symptoms and history.
- Use of this assay should only be for trained personnel.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute.
- When processing more than one sample at a time, open only one cartridge; add the Sample Reagent-treated sample and close the cartridge before processing the next sample. Change gloves between samples
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute Xpert Ebola Assay reagents with other reagents.
- Do not open the Xpert Ebola Assay cartridge lid except when adding the Sample Reagent-treated sample.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Ebola Assay cartridge is used to process one test. Do not reuse spent cartridges.
- The single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes.
- The single-use disposable swab is used to collect and/or transfer one specimen. Do not reuse spent disposable swabs.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO (World Health Organization) medical waste handling and disposal guidelines.
- Store the Xpert Ebola Assay kit at 2–28 °C.

Note Before starting, remove the bottle containing the Sample Reagent from the kit and allow to adjust to room temperature. See Figure 1. If the bottle has not been stored in an upright position, make sure the buffer is settled in the bottom by giving the bottle a firm shake.

Note Wear disposable gloves. Label the Sample Reagent vial with the specimen identification.

10 Chemical Hazards[1,2]

- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - May be harmful in contact with skin
  - Causes eye irritation
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

11 Specimen Collection, Transport, and Storage

11.1 Whole Blood

Collection

Collect whole blood specimens by venipuncture in EDTA tubes per the manufacturer's instructions for use. A minimum of 100 µL of whole blood is required for the Xpert Ebola Assay.

Important **Immediately proceed with the sample preparation step to ensure that the Ebola virus gets inactivated.**

Sample Preparation

Venous Whole Blood collected in EDTA-tubes: Open the lid of the Sample Reagent bottle. Transfer 0.1 ml blood by placing the swab (SWAB/E-50) in the EDTA tube and allow it to absorb blood for at least 30 seconds, transfer the sample by inserting the prepared swab into the Sample Reagent bottle (see Figure 1). Hold the swab by the stem and align the small groove against the rim of the tube. Break off the swab by bending to one side.

Note Use sterile gauze to minimize risk of contamination.

Close the lid of the Sample Reagent bottle and mix the sample by vortex for 10 seconds. Let it incubate at room temperature for 20 minutes.

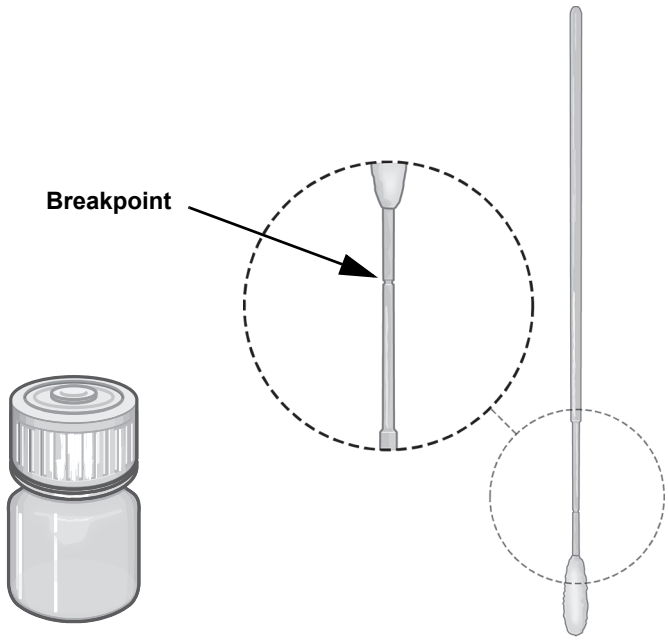

Figure 1. Xpert Ebola Assay Sample Reagent Bottle and Ebola Sample Collection Swab

11.2 Sample Transport and Storage

Transport Sample Reagent-treated samples to the testing laboratories for further processing in individual resealable bags according to WHO guidelines for transport of Ebola specimens, "How to safely collect blood samples from persons suspected to be infected with highly infectious blood-borne pathogens (e.g. Ebola)". The Sample Reagent-treated blood samples may be stored for up to 72 hours at 2-8 °C and for up to 48 hours at 8-30 °C or for up to 24 hours at 28-35 °C.

12 Procedure

12.1 Preparing the Cartridge

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

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Wear protective disposable gloves.
2. Label the Sample Reagent vial with the specimen identification.
3. Inspect the test cartridge for damage. If damaged, do not use.
4. Open the cartridge lid.
5. Use the 1 mL transfer pipette (see Figure 2) or an automatic pipette to transfer 1 mL of the sample reagent-treated specimen into the sample chamber of the cartridge (see Figure 3). Do **NOT** pour the specimen into the chamber.

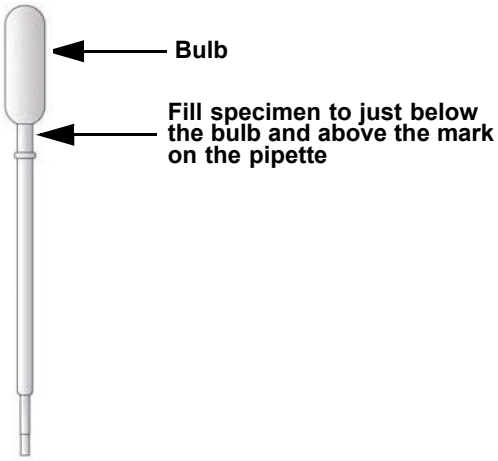

Figure 2. Xpert Ebola Assay 1 mL Transfer Pipette

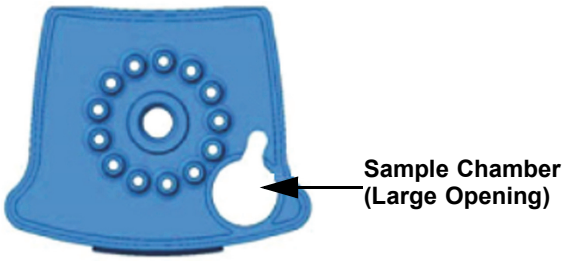

Figure 3. Xpert Ebola Assay Cartridge (Top View)

12.2 Starting the Test

Important **Before starting the test, make sure the Xpert Ebola Assay Definition File is imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

1. Turn on the GeneXpert instrument system:
   - If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. The Xpertise software will launch automatically or may require double clicking the Xpertise software shortcut icon on the Windows desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (Infinity).
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all reports. The Scan Cartridge dialog box appears.
6. Scan the barcode on the Xpert Ebola Assay cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN.
7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). Enter your password, if requested.
8. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

   or

   For the GeneXpert Dx Instrument:

   A. Open the instrument module door with the blinking green light and load the cartridge.

   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.

   C. Wait until the system releases the door lock before opening the module door. Then remove the cartridge.

   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

13 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual,* depending on the instrument used.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

14 Quality Control

CONTROL

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

- **Sample Adequacy Control (SAC)**: Ensures that the sample was correctly added to the cartridge. The SAC verifies that the correct in-volume of sample has been added in the sample chamber. The SAC passes if it meets the validated acceptance criteria.
- **Sample Processing Control (SPC)**: Ensures the sample was correctly processed. The SPC is an Armored RNA® control in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample virus. The SPC verifies that lysis of Ebola has occurred if the organism is present and verifies that the specimen processing is adequate. Additionally this control detects specimen-associated inhibition of the RT-PCR reaction. 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.
- **Probe Check Control (PCC)**: Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.
- **External Controls**: External controls should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable.
- Negative venous whole blood specimens can be used as External Negative Controls to be run as patient specimens. For information on how to obtain optional external control materials, contact Technical Support at TechSupport@cepheid.com or www.cepheid.com under the **SUPPORT** tab.

15 Interpretation of Results

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window (see Figure 4, Figure 5, Figure 6 and Figure 7). Possible results are shown in Table 1.

Table 1. Xpert Ebola Assay Results and Interpretation

Result	Interpretation
Ebola GP DETECTED, Ebola NP DETECTED **or** **Ebola GP DETECTED, Ebola GP NOT DETECTED** **or** **Ebola GP NOT DETECTED, Ebola NP DETECTED** See Figure 4, Figure 5 and Figure 6.	The EBOLA target nucleic acids are detected. • The EBOLA signal for both or one of the two nucleic acids target have a Ct within the valid range and endpoint above the minimum setting. • SAC: NA (not applicable); SAC is ignored because the EBOLA target amplification occurred. • SPC: NA (not applicable); SPC is ignored because the EBOLA target amplification occurred. • Probe Check: PASS; all probe check results pass.
Ebola GP NOT DETECTED, Ebola NP NOT DETECTED See Figure 7.	The EBOLA target nucleic acids are not detected. SPC meets acceptance criteria. • SAC: PASS; SAC has a Ct within the valid range and endpoint above the minimum setting. • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check: PASS; all probe check results pass.

Table 1. Xpert Ebola Assay Results and Interpretation (Continued)

Result	Interpretation
INVALID	Presence of absence of the target nucleic acids cannot be determined. Repeat test according to instructions in Retest Procedure. • SAC: FAIL; SAC Ct is not within the valid range and the endpoint is below the minimum setting. • SPC: PASS; SPC has a Ct within the valid range and the endpoint above the minimum setting. • Probe Check: PASS; all probe check results pass. Or • SAC: PASS; SAC has a Ct within the valid range and the fluorescence endpoint above the minimum setting. • SPC: FAIL; SPC Ct is not within the valid range and the endpoint is below the minimum setting. • Probe Check: PASS; all probe check results pass.
ERROR	Presence or absence of EBOLA nucleic acids cannot be determined. Repeat test according to the instructions in Retest Procedure. • EBOLA: NO RESULT • SAC: NO RESULT • SPC: NO RESULT • Probe Check: FAIL, all or one of the probe checks fail.
NO RESULT	Presence or absence of EBOLA target nucleic acids cannot be determined. Repeat test according to the instructions in Retest Procedure. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • EBOLA: NO RESULT • SAC: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

Note Assay screenshots are for example only and may vary from screenshots shown in this package insert. QC1 and QC2 in legends of Figure 4, Figure 5, Figure 6, and Figure 7 control for presence of probes (see Probe Check Control in Section 14, Quality Control); amplification curves not generated.

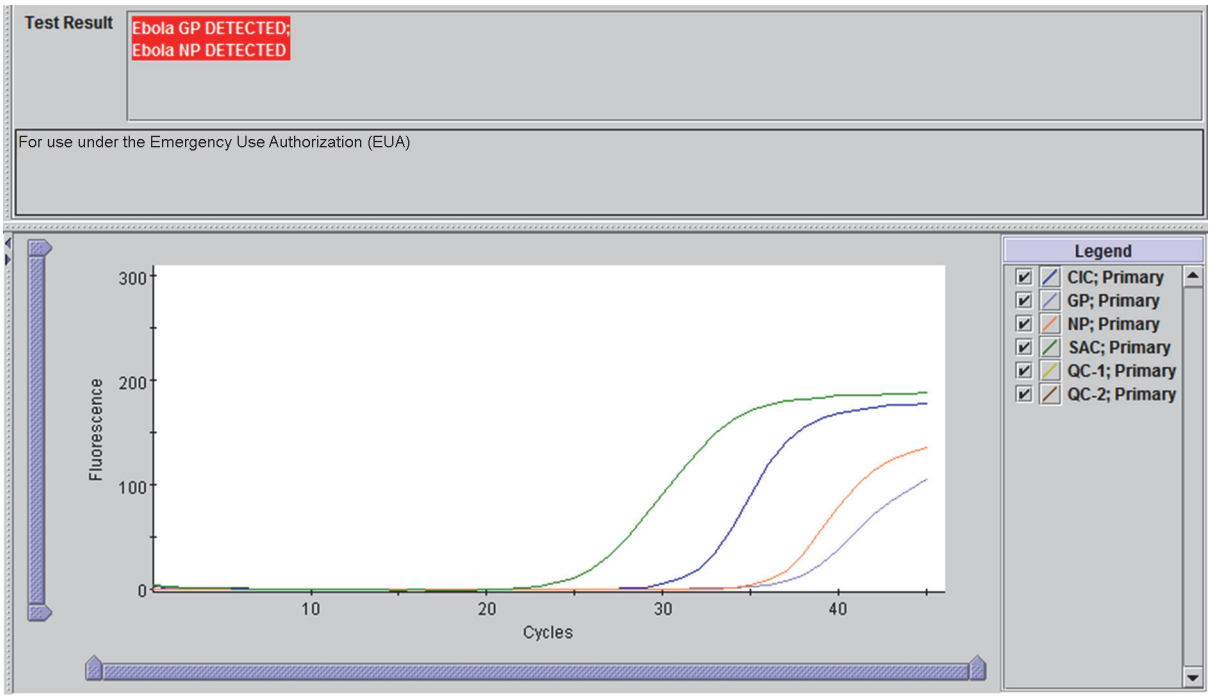

Figure 4. Ebola GP DETECTED, Ebola NP DETECTED

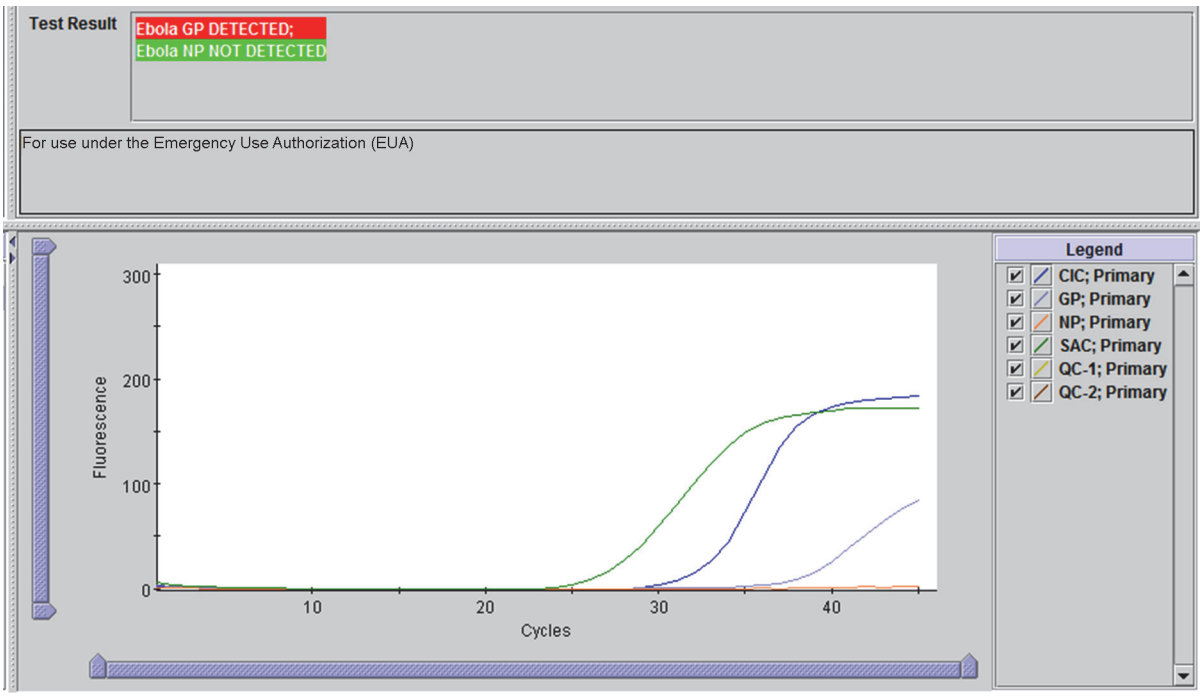

Figure 5. Ebola GP DETECTED, Ebola NP NOT DETECTED

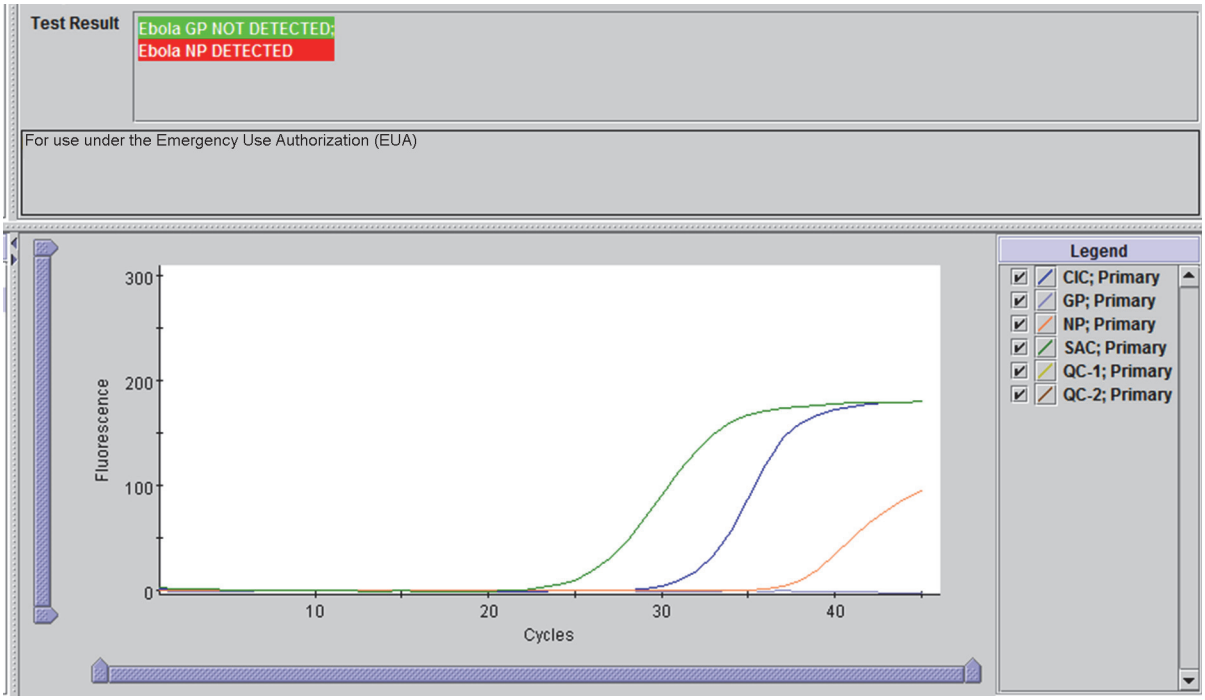

Figure 6. Ebola GP NOT DETECTED, Ebola NP DETECTED

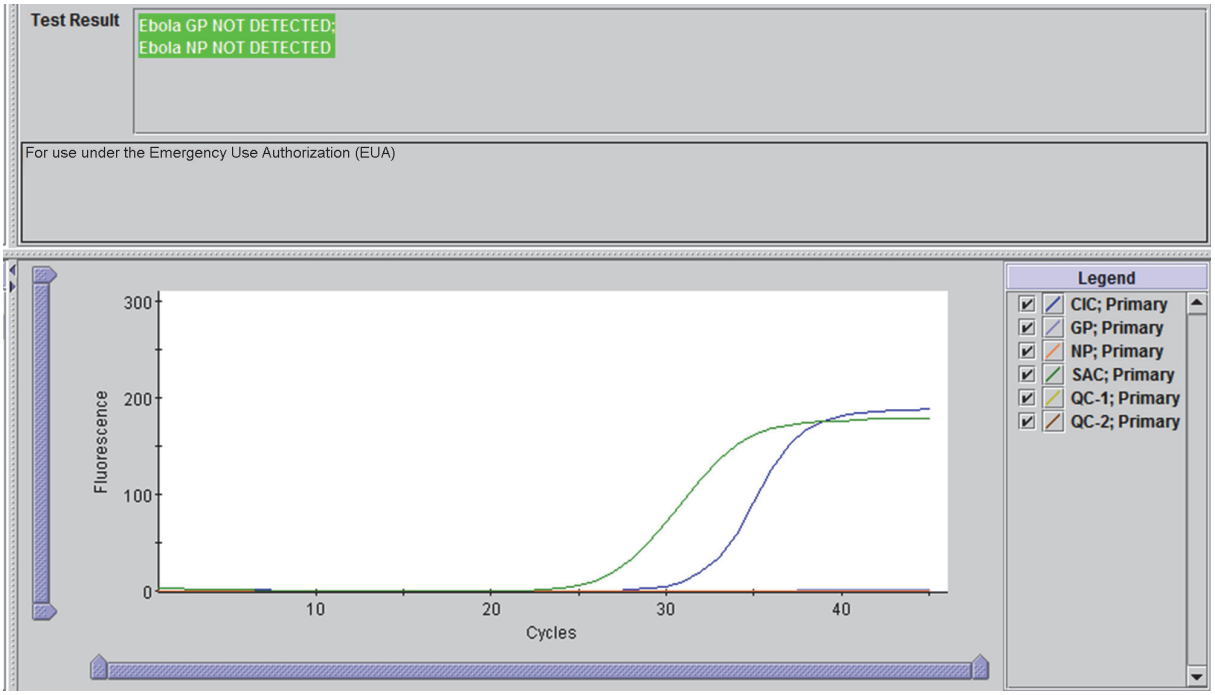

Figure 7. Ebola GP NOT DETECTED, Ebola NP NOT DETECTED

16 Retests

16.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test according to the instructions in Section 15.2 Retest Procedure.

- An **INVALID** result indicates one or more of the following
  - The control SPC failed.
  - The sample was not properly processed or PCR is inhibited.
  - The control SAC failed.
  - The added sample volume was insufficient.
- An **ERROR** result indicates that the assay was aborted. Possible causes include: the reaction tube being filled improperly, a reagent probe integrity problem was detected, because the maximum pressure limit was exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or a power failure occurred.

16.2 Retest Procedure

For retest of a **NO RESULT**, **INVALID**, or **ERROR** result, use a new cartridge (do not re-use the cartridge) and new reagents.

1. Remove a new cartridge from the kit.
2. See Section 12.1, Preparing the Cartridge, and Section 12.2, Starting the Test.

17 Performance Characteristics

17.1 Limit of Detection

The limit of detection (LoD) of the Xpert Ebola Assay was estimated for Ebola Zaire RNA and for live Ebola Zaire virus. Testing was performed with three dilution panels each tested using one reagent kit lot. Viral RNA purified from Ebola Zaire Mayinga virus obtained from Public Health Agency of Sweden was diluted in a mixture of Sample Reagent and whole blood and the live Ebola virus 2014/Gueckedou-C05 and 2014/Gueckedou-C07 was each diluted in EDTA whole blood. In total, 20 RNA replicates and 4 live virus replicates per level and specimen were tested. The LoD using RNA was estimated as the lowest concentration of target Ebola Zaire RNA that could be reproducibly distinguished from negative samples with 95% probability using Probit analysis. Verification of the estimated LoD of Ebola RNA was performed on one reagent lot with 25 replicates. All 25 replicates were positive. The estimated LoD of live virus was confirmed as the lowest concentration of plaque forming unit (PFU) per mL EDTA whole blood at which at least 19 out of 20 replicates were positive. The results for Ebola Zaire RNA and live virus are shown in Table 2 and Table 3.

Table 2. Limit of Detection for Ebola Zaire RNA for Xpert Ebola Assay Using Probit Regression

Specimen	Nominal Concentration (copies/mL)	Total Replicates (N)	Total Positives (N)	Positivity Rate (%)	LoD with 95% Probability Estimated by Probit (95% Confidence Interval)
Ebola Zaire Mayinga RNA	700	20	20	100	232.4 copies/mL (95% CI 163.1-301.6)
	300	20	20	100	
	150	20	13	65	
	75	20	12	60	
	30	20	9	45	
	15	20	5	25	

Table 3. Numbers of Positive Replicates Per Level for Ebola Zaire Makona-Gueckedou 07 and 05 Virus in EDTA-WB and Confirmation of Limit of Detection

Specimen	Nominal Concentration (PFU/mL)	Total Replicates (N)	Total Positives (N)	Positivity Rate (%)	Confirmation of LoD		
					Nominal Concentration (PFU/mL)	Total Replicates (N)	Total Positives (N)
Ebola Zaire Makona-Gueckedou 07 virus	50	4	4	100	1.0	20	20
	25	4	4	100			
	12.5	4	4	100			
	1	4	4	100			
	0.1	3	1	33			
	0.01	4	0	0			
Ebola Zaire Makona-Gueckedou 05 virus	0.13	4	4	100	0.13	20	20
	0.065	4	4	100			
	0.0325	4	3	75			
	0.01625	4	1	25			

17.2 Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the Xpert Ebola was determined for four Ebola Zaire strains other than Mayinga that were available in the form of live Ebola virus or viral RNA. In addition, *in silico* analysis of all other, not tested, available sequences of Ebola Zaire strains was performed. The test samples were prepared by spiking each individual specimen into Ebola negative EDTA whole blood, or if RNA prepared from virus was used, into Ebola negative EDTA whole blood mixed with Sample Reagent (SR). Each specimen was tested in replicates of 20 and a negative control specimen, comprised of Ebola negative EDTA whole blood, was tested in replicates of three using one kit lot of reagents. The test results for Ebola positive specimens are presented in Table 4. All Ebola negative control specimens were reported **Ebola GP NOT DETECTED, Ebola NP NOT DETECTED**.

Table 4. Analytical Reactivity for the Xpert Ebola Assay

Ebola Zaire Strain	Specimen Type	Testing Concentration	Total Replicates (N)	Total Positives (N)	Positivity Rate (%)
Guinea	Live virus	1x LoD	20	20	100%
Ekron	Live virus	3x LoD	20	20	100%
Gabon	Live virus	3x LoD	20	20	100%
Kikwit	RNA	5x LoD	20	20	100%

In silico analysis was performed to predict the performance of the Xpert Ebola Assay in detection of all Ebola Zaire variant sequences available in GenBank; from the first Zaire sequence data published in 1976 to the sequences from the current West Africa outbreak. The two Xpert Ebola amplicon sequences derived from Zaire glycoprotein (GP) and nucleoprotein (NP) genes were each submitted to BLAST (NCBI). Also, all six Xpert Ebola oligo sequences were checked individually against a local database alignment containing all Ebola Zaire sequences available in GenBank. The analyses show that the Ebola Zaire NP and GP oligonucleotides completely match all Zaire sequences present in GenBank.

17.3 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Ebola Assay was evaluated by testing non-Ebola viruses and bacteria and non-Zaire Ebola strains at clinically relevant levels. The specimens were prepared by spiking each individual organism into Ebola negative EDTA whole blood or if genomic RNA/DNA of the organism was used, into Ebola negative EDTA whole blood mixed with Sample Reagent. The analytical specificity test results are shown in Table 5 and Table 6. The analytical specificity of the Xpert Ebola Assay for the evaluated organisms is 100%.

Table 5. Analytical Specificity Determination for Xpert Ebola Assay, non-Zaire Ebola Positive Specimens

Organism	Specimen Type	Testing Conc. (Particle Conc. Used for Nucleic Acid Isolation)	Unit (ng or PFU/mL WB)	N	Positive Results	Negative Results
Ebola Ivory Coast	Nucleic acids	546[a]	ng/mL	3	0	3
Ebola Reston	Nucleic acids	$3.0x10^5$	PFU/mL	3	0	3

a. RNA concentration of the stock material

Table 6. Analytical Specificity Determination for Xpert Ebola Assay, non-Ebola Specimens

Organism	Specimen Type	Testing Conc. (Particle Conc. Used for Nucleic Acid Isolation)	Unit (ng or PFU/mL WB)	N	Positive Results	Negative Results
Chikungunya Virus (181/25)	Nucleic acids	2798[a]	ng/mL	3	0	3
Coxiella burnetti	Nucleic acids	50	ng/mL	3	0	3
Crimean Congo Hemorrhagic Fever virus (Dubai)	Nucleic acids	$3.4x10^6$	PFU/mL	3	0	3
Dengue virus (Type 2)	Nucleic acids	$2.7x10^6$	PFU/mL	3	0	3
Hemophilus influenzae	Nucleic acids	50	ng/mL	3	0	3
Influenza virus A (H9N2)	Nucleic acids	$1.0x10^5$	PFU/mL	3	0	3
Lassa virus (Pinneo)	Nucleic acids	$5.7X10^3$	PFU/mL	3	0	3
Marburg (Angola)	Nucleic acids	$2.6x10^6$	PFU/mL	3	0	3
Marburg (Angola)	Live virus	$5.0x10^{4b}$	PFU/mL	3	0	3
Marburg (Musoke)	Nucleic acids	$6.0x10^4$	PFU/mL	3	0	3
Marburg (Musoke)	Live virus	$5.0x10^{4b}$	PFU/mL	3	0	3
Marburg (Ravn)	Nucleic acids	$4.8x10^5$	PFU/mL	3	0	3
Mosquito	Nucleic acids	50	ng/mL	3	0	3
Pseudomonas aeruginosa	Nucleic acids	50	ng/mL	3	0	3
Rickettsia conorii	Nucleic acids	50	ng/mL	3	0	3
Rickettsia prowazekii	Nucleic acids	50	ng/mL	3	0	3
Rickettsia typhi	Nucleic acids	50	ng/mL	3	0	3
Rift Valley Fever virus (SA51)	Nucleic acids	$7.5X10^5$	PFU/mL	3	0	3
Salmonella bongori	Nucleic acids	50	ng/mL	3	0	3
Salmonella typhi	Nucleic acids	50	ng/mL	3	0	3

Table 6. Analytical Specificity Determination for Xpert Ebola Assay, non-Ebola Specimens (Continued)

Organism	Specimen Type	Testing Conc. (Particle Conc. Used for Nucleic Acid Isolation)	Unit (ng or PFU/mL WB)	N	Positive Results	Negative Results
Shigella flexneri Type2	Nucleic acids	50	ng/mL	3	0	3
Streptococcus pneumoniae	Nucleic acids	50	ng/mL	3	0	3
Tick	Nucleic acids	50	ng/mL	3	0	3
Yellow fever (OBS-6745)	Nucleic acids	$1.0x10^6$	PFU/mL	3	0	3
Yersinia enterocolitica	Nucleic acids	50	ng/mL	3	0	3
Yersinia pestis	Nucleic acids	50	ng/mL	3	0	3

a. RNA concentration of the stock material
b. Testing concentration of live virus.

In silico analysis were performed to predict the risk of cross reactivity of the Xpert Ebola Assay Zaire target oligonucleotides (GP and NP) to non-Zaire Ebola viruses, as well as towards all the exclusivity disease pathogens listed in Table 7. The analyses show that the Xpert Ebola primer and probe sequences are specific and should not yield false positive Ebola Zaire results with the evaluated organisms.

Table 7. Analytical Specificity *In Silico* Analysis Organisms

Organism
Ebola Sudan-Boniface
Ebola Sudan-Bunidbugyo
Ebola Sudan-Gulu
Adenovirus
Borrelia recurrentis
Enterovirus
Influenza virus B
Leptospira genus
Marburg (Ci67)
Neisseria meningitidis
Plasmodium falciparum
Plasmodium malariae
Plasmodium ovale
Plasmodium vivax
Rickettsia africae
Rotavirus
RSV
Trypanosoma
Vibrio cholera

17.4 Potentially Interfering Substances

The susceptibility of the Xpert Ebola Assay to interference by elevated levels of endogenous substances encountered in whole blood was evaluated. For endogenous substances, Ebola negative EDTA whole blood and Ebola positive EDTA whole blood spiked with the substances were tested. To prepare Ebola positive specimens, Ebola Zaire Mayinga RNA (2,500 copies/mL) was added to the Sample Reagent which then was mixed with EDTA whole blood spiked individually with each interfering substance. A total of five substances were evaluated at concentrations shown in Table 8. Six replicates of each specimen were tested using one reagent kit lot. Elevated levels of the endogenous substances listed in Table 8 were shown not to impact the assay specificity or interfere with the Ebola detection.

Table 8. Endogenous Substances and Concentration Tested

Endogenous Substances	Concentration Tested
Albumin	90.0 mg/mL
Bilirubin	0.300 mg/mL
Human DNA	4.0 µg/mL
Hemoglobin	5.0 mg/mL
Triglycerides	30.0 mg/mL

17.5 Contrived Clinical Specimens Testing

Performance characteristics of the Xpert Ebola Assay were evaluated using mock clinical specimens. Due to the difficulty of obtaining clinical specimens from Ebola infected patients, mock specimens were prepared by spiking Ebola live virus or Ebola viral RNA into EDTA-whole blood (WB) specimens obtained from different Ebola negative individuals. The WB was spiked with Ebola virus or viral RNA in varying concentrations from near the LoD to high levels (up to 200x the limit of detection [LoD]). In addition, un-spiked EDTA-WB specimens from different individual negative donors were also tested. Specimens were blinded when tested with the Xpert Ebola Assay.

The positive percent agreement for EBOV Mayinga RNA was 100.0% (50/50, [95% CI: 92.9-100.0]), for Makona-Gueckedou 05 live virus was 100.0% (50/50, [95% CI: 92.9-100.0]), and for Makona-Gueckedou 07 live virus was 84.0% (42/50, [95% CI: 71.5-97.1]). The negative percent agreement was 100.0% (50/50 [97.5% CI 92.9-100.0]) for each study. Table 9, Table 10, and Table 11 show the results for both the negative and the Ebola spiked specimens.

Table 9. Numbers of Positive and Negative Test Results for Ebola Zaire Mayinga RNA Spiked Specimens and Negative Control Specimens

Nominal Concentration	N	Positive Results		Negative Results
0	50	0		50
1xLoD	25	25		0
3xLoD	10	10		0
10xLoD	10	10		0
100xLoD	5	5		0
				95% CI
Positive Percent Agreement		50/50	100%	92.9%-100%
Negative Percent Agreement		50/50	100%	92.9%-100%

Table 10. Numbers of Positive and Negative Test Results for Ebola Makona-Gueckedou 05 Virus Spiked Specimens and Negative Control Specimens

Nominal Concentration	N	Positive Results		Negative Results
0	50	0		50
1xLoD	25	25		0
3xLoD	10	10		0
10xLoD	10	10		0
100xLoD	5	5		0
				95% CI
Positive Percent Agreement		50/50	100%	92.9%-100%
Negative Percent Agreement		50/50	100%	92.9%-100%

Table 11. Numbers of Positive and Negative Test Results for Ebola Makona-Gueckedou 07 Virus Spiked Specimens and Negative Control Specimens

Nominal Concentration	N	Positive Results		Negative Results
0	50	0		50
2xLoD	25	21		4
6xLoD	10	10		0
20xLoD	10	6		4
200xLoD	5	5		0
				95% CI
Positive Percent Agreement		42/50	84.0%	71.5%-97.1%
Negative Percent Agreement		50/50	100%	92.9%-100%

Investigation of the difference in the PPA results for the contrived Ebola Makona-Gueckedou 07 Virus spiked specimens (Table 11) compared to the other two contrived sets (Table 9 and Table 10) of specimens showed inconsistencies in specimen preparation. Swabs were not completely immersed in the specimens containing the whole blood specimens spiked with Ebola Makona-Gueckedou 07 limiting the amount of sample available for testing. The testing for the contrived Ebola Makona-Gueckedou 07 Virus spiked specimens was repeated using 50 individual WB specimens at the correct final concentrations and volume for each specimen. Table 12 shows the summary results at each concentration tested and the positive and negative percent agreement for the repeated study.

Table 12. Summary of Results and Positive and Negative Percent Agreement for Mock Clinical Specimens Spiked with Ebola Makona-Gueckedou 07 virus—Texas

Nominal Concentration	N	Positive Results		Negative Results
0	6	0		6
1xLoD	25	20		5
3xLoD	10	10		0
10xLoD	10	10		0
100xLoD	5	5		0
				95% CI
Positive Percent Agreement		45/50	90.0%	78.6%-95.7%
Negative Percent Agreement		6/6	100%	61.0%-100%

18 Assay Limitations

- Negative test results do not preclude Ebola virus infection and should not be used as the sole basis for treatment or other patient management decisions
- All test results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms.
- This test has been evaluated for use with human venous whole blood only.
- Specimens from patients who have received therapeutics or vaccines based on nucleic acid sequences derived from Ebola Zaire virus may exhibit false positive or other confounding test results.
- This test is a qualitative test and does not provide a quantitative value for the virus in the sample.
- Interpretation of results from the Xpert Ebola Assay must account for the possibility of false-positive and false-negative results.
- False positive results may occur from cross-contamination by target organism, their nucleic acids, or from PCR amplicon.
- Failure to follow assay procedures may lead to false results.
- Inhibitors present in the samples may lead to false-negative results.
- Erroneous test results might occur from improper specimen collection, handling, storage, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance to the instructions in this package insert is necessary to avoid erroneous results.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants and may result in a false negative result.

19 References

1. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC).
- Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z)

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21 Technical Assistance

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22 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	For in vitro diagnostic use
EUA	For use under Emergency Use Authorization (EUA) Only/ Emergency Use Authorization
2	Do not reuse
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
Σ	Contains sufficient for <n> tests
CONTROL	Control
°C	Temperature limitation
	Biological risks
	Expiration date

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IVD

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