GeneXpert
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GeneXpert® Ebola Assay

REF **REBOLA-50**

Cepheid® **For Research Use Only (RUO)**

RUO

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GeneXpert® Ebola Assay

For Research Use Only (RUO).

1 Proprietary Name

GeneXpert® Ebola Assay

2 Common or Usual Name

GeneXpert Ebola Assay

3 Description

The GeneXpert Ebola Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of RNA from the Ebola Zaire virus from whole blood and buccal cheek swabs.

4 Summary and Explanation

Ebola virus disease (EVD) has occurred sporadically throughout West Africa for decades of outbreak, 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'Ivoire (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 GeneXpert 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 or buccal swab sample types.

5 Principle of the Procedure

The GeneXpert 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 GeneXpert Ebola Assay includes reagents for the detection of Zaire Ebola virus total nucleic acids in samples 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 GeneXpert Ebola Assay kit contains sufficient reagents to process 50 samples or quality control samples. The kit contains the following:

GeneXpert Ebola Assay Cartridges with Integrated Reaction Tubes	**50 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 (Sample Reagent)	**50 bottles per kit**
• Lysis Reagent (Guanidinium Thiocyanate)	50 x 2.5 mL per bottle
Disposable 1 mL Transfer Pipettes	**50 per kit**
Disposable Swabs (catalog # SWAB/E-50)	**1 bag, 50 swabs**
CD	
Instructions for Use (Package Insert)	

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

7 Storage and Handling

- Store the GeneXpert 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 4.3 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.
- Chlorine Bleach

9 Warnings and Precautions

- 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 GeneXpert Ebola Assay reagents with other reagents.
- Do not open the GeneXpert 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 GeneXpert 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 GeneXpert 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 sample identification.

10 Chemical Hazards[1, 2]

- WARNING
- **Hazard Statements**
  - Harmful if swallowed
  - May be harmful in contact with skin
  - Causes eye irritation
- **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.
  - **Storage/Disposal**
    - Dispose of contents and/or container in accordance with local, regional, national, and/or international regulations.

11 Sample Collection, Transport, and Storage

11.1 Whole Blood

Collection

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

Alternatively, use the swab (SWAB/E-50) to collect blood samples from a finger stick. Allow at least 2/3 of swab head to absorb blood. Immediately transfer the swab into the tube containing the sample reagent (see Figure 1 and under sample preparation).

11.2 Buccal Swab

Use the swab (SWAB/E-50) to collect buccal swab samples according to WHO guidance for collection of oral swabs for Ebola testing*. Avoid touching the swab tip with the gloves or against any surface.

For living subjects, place the swab in the opened mouth and immediately bring the swab tip to inside of cheek and rub firmly with circular motions and solid pressure for at least 20 seconds with the entire swab head. For deceased subjects, place the palm of hand onto the chin and press down firmly to open the mouth slightly. Insert the swab into the side of the cheek and rub firmly with circular motions and solid pressure for at least 20 seconds with the entire swab head. Repeat for the other cheek.

*WHO reference number: WHO/EVD/Guidance/Lab/14.2

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

Sample Preparation

Blood collected in EDTA-tubes: Open the lid of the sample reagent bottle. Transfer 100 µL blood by placing the swab (SWAB/E-50) in the EDTA tube and allow it to absorb blood for at least 5 seconds, transfer the sample by inserting the prepared swab into the tube containing the Sample Reagent (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. Alternatively, use a calibrated pipette with filtered disposable tip to transfer 100 µL of blood into the tube containing the Sample Reagent (see Figure 1).

Buccal Swab: Open the lid of the sample reagent bottle. Insert the prepared swab into the Sample Reagent (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.

Blood collected from finger stick: Open the lid of the sample reagent bottle. Insert the prepared swab into the tube containing the Sample Reagent (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.

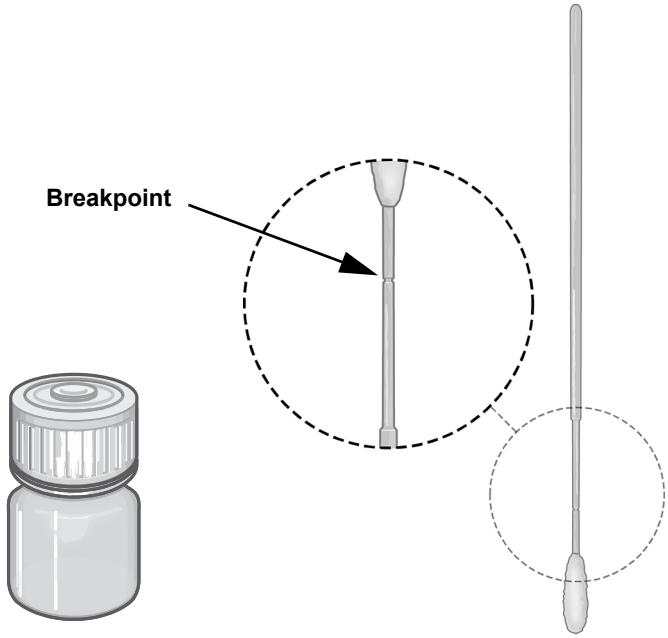

Figure 1. GeneXpert Ebola Assay Sample Reagent Bottle and Ebola Sample Collection Swab

11.3 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 samples, "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 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 sample 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 sample into the sample chamber of the cartridge (see Figure 3). Do **NOT** pour the sample into the chamber.

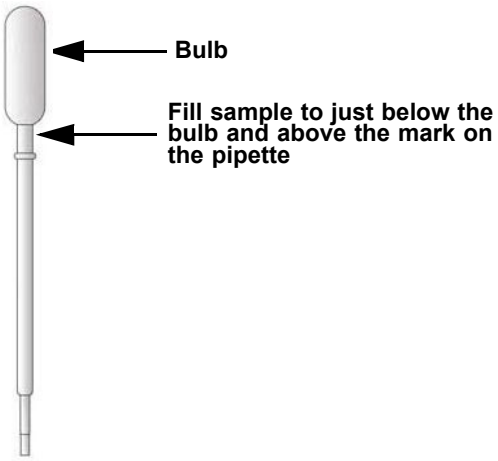

Figure 2. GeneXpert Ebola Assay 1 mL Transfer Pipette

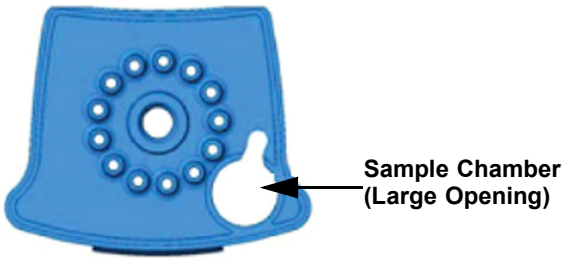

Figure 3. GeneXpert Ebola Assay Cartridge (Top View)

12.2 Starting the Test

Important **Before starting the test, make sure the GeneXpert 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 subject ID (optional). If typing the subject ID, make sure the subject ID is typed correctly. The subject 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 GeneXpert 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 sample 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)**: The SAC ensures that human cells have 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 sample processing is adequate. Additionally, this control detects sample-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, QC1, QC2)**: Before the start of the reverse transcription PCR assay, the GeneXpert Instrument System measures the fluorescence signal from the FAM-2-GP and CF-5-SPC probes (denoted as QC1 and QC2) to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Because QC1 and QC2 are measured at the time of reverse transcription PCR step (prior to the real-time PCR step), growth curve is not available. The PCC passes if it meets the assigned acceptance criteria.
- **External Controls**: External controls should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable.

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. GeneXpert 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.
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 Ct is within the valid range and the endpoint is 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.

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

Result	Interpretation
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.

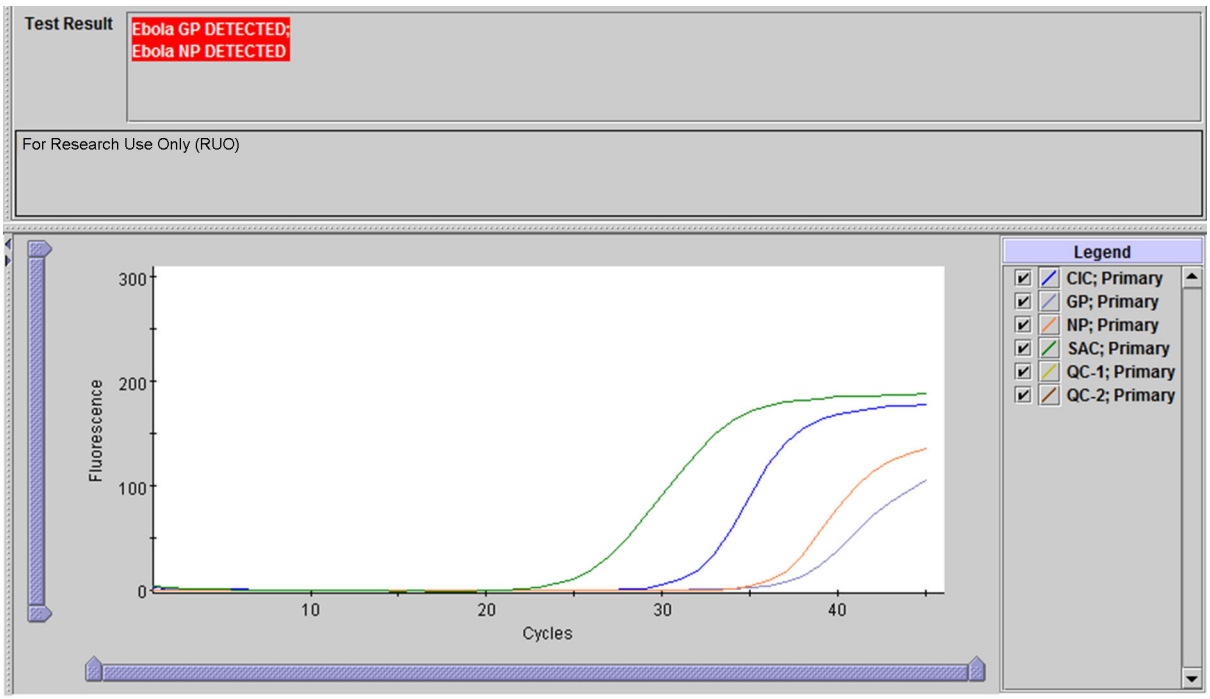

Figure 4. Ebola GP DETECTED, Ebola NP DETECTED

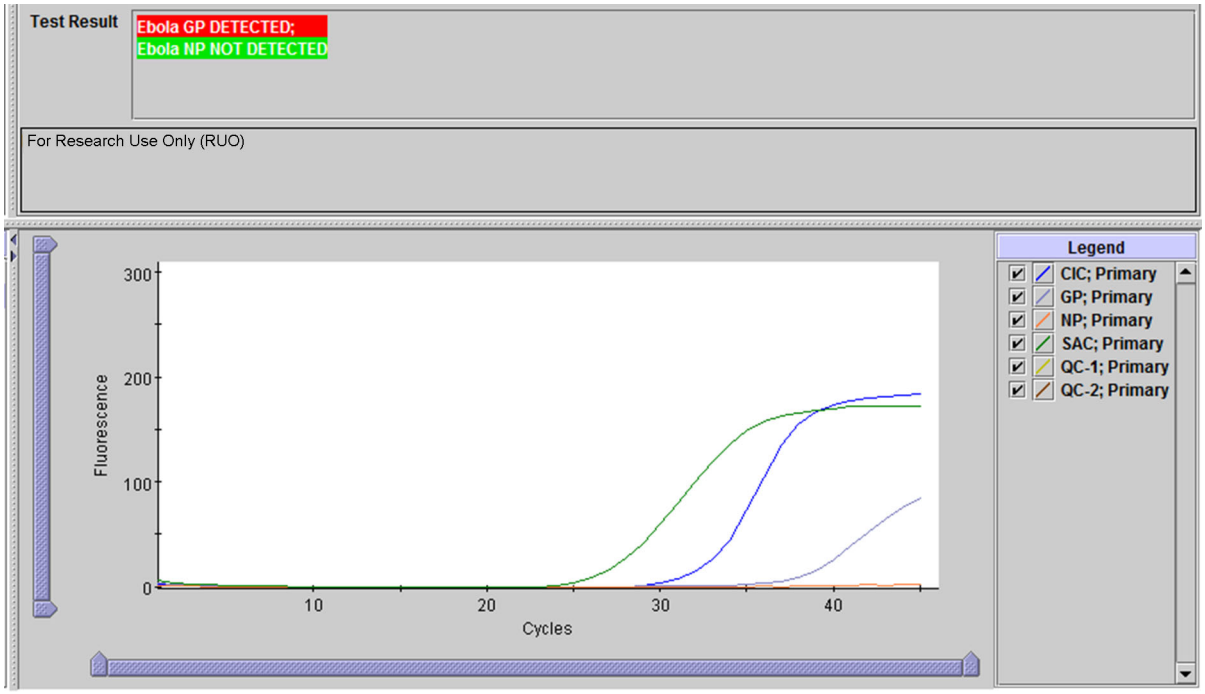

Figure 5. Ebola GP DETECTED, Ebola NP NOT DETECTED

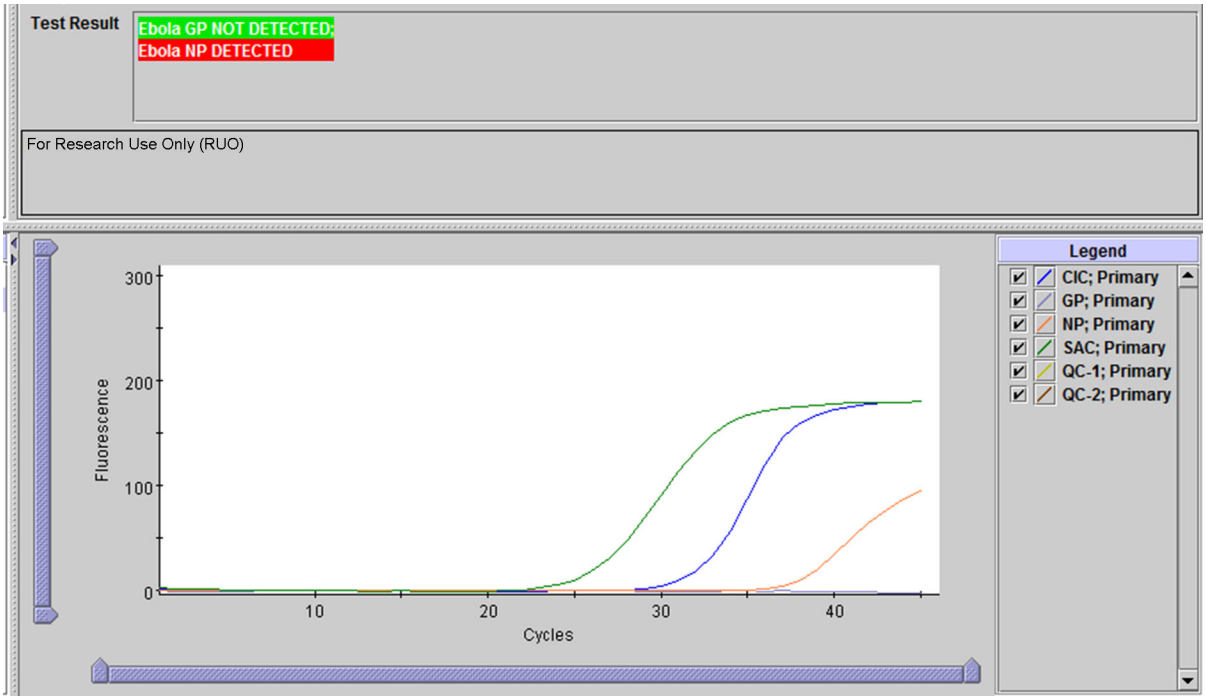

Figure 6. Ebola GP NOT DETECTED, Ebola NP DETECTED

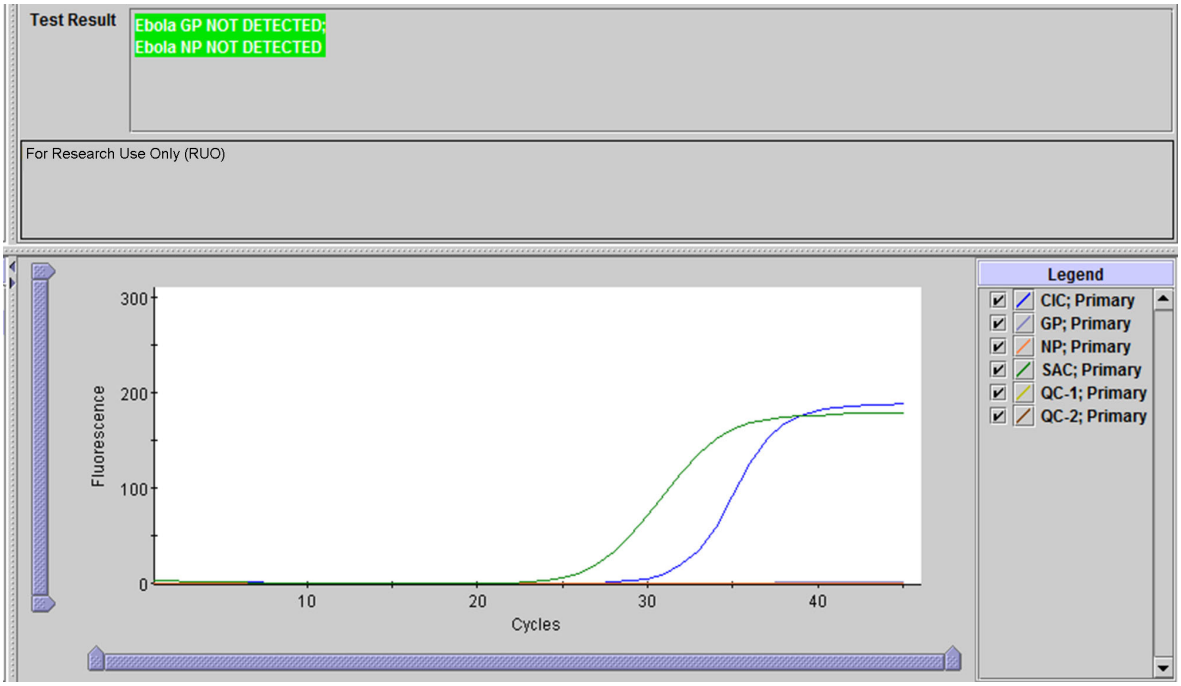

Figure 7. Ebola GP NOT DETECTED, Ebola NP NOT DETECTED

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 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) No 1907/2007).
2. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

18 Cepheid Headquarters Locations

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19 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

Contact Information

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Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/CustomerSupport.

20 Table of Symbols

Symbol	Meaning
REF	Catalog number
RUO	For research use only (RUO)
2	Do not reuse
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
CC	Country of manufacture
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning

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RUO

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