

Customer Technical Training

Xpert[®] Ebola

For CE-IVD Use Only



Training Agenda

- **Xpert® Ebola***
 - Intended use
 - Reagents
 - Sample collection
 - Kit storage and handling
 - Cartridge preparation
 - Quality Controls
 - Results analysis
- **Discussion**



The Cepheid Solution



- Dual-target design: Each target covering known Zaire strains
 - Nucleoprotein (NP)
 - Glycoprotein (GP)
- On-board controls for each individual sample
 - Probe Check Control (PCC)
 - Sample Adequacy Control (SAC)
 - Cepheid Internal Control (CIC)
- Closed cartridge system minimizes risk of contamination
- On-demand results 24/7
- Random access

Intended Use

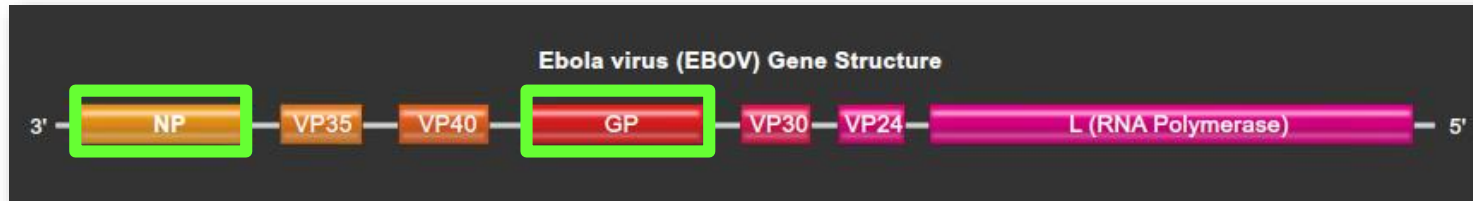
The Xpert® Ebola* Assay is a RT-PCR test intended for the qualitative detection of RNA from the **Ebola Zaire virus** in EDTA venous whole blood, peripheral blood from finger-stick, or buccal swab from individuals **with signs and symptoms** of Ebola Virus Disease (EVD) in conjunction with **epidemiological 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.

Targets and Probes

- Qualitative real-time reverse transcription polymerase chain reaction (RT-PCR) test for the detection of RNA from the Ebola Zaire virus
- **Detection of Nucleoprotein (NP) and Glycoprotein (GP) gene sequences**



- **4 probes (NP, GP, CIC and SAC)**

Assay Requirements

GeneXpert Systems

- GeneXpert Dx Software **v4.4 a** or higher
- Xpertise Software **v6.2** or higher

Test Kits (CE-IVD)*

- GXEBOLA-CE-50

Materials Required but not Provided

- Disposable lancets for finger prick
- Disposable swabs *SWAB/E-50*
- Vortex
- Personal Protective Equipment (PPE)
- 1:10 Bleach
- 70% ethanol or denatured ethanol

Optional

- Uninterruptible Power Supply /Surge Protector
- Printer

Good Laboratory Practice

Personel Protective Equipment (PPE)

- Wear clean lab coats, safety glasses, and gloves
- Change gloves between processing samples

Lab Bench area

- Clean work surfaces routinely with:
 - ✓ A final concentration of 1:10 dilution of household chlorine bleach (used within 1 day of preparation)*
 - ✓ 70% Ethanol or denatured Ethanol
- * Please refer to the GeneXpert Operator Manual for details*
- After cleaning, ensure work surfaces are dry

Specimens, Samples, and Kits Storage

- Store specimens and sample away from kit to prevent contamination

Equipment(s)

- Use filtered pipette tips when recommended
- Follow the manufacturer's requirements for calibration and maintenance of equipment(s)

Kit Handling



Xpert® Ebola* Kit Content

Xpert® Ebola Assay	
Catalog Number	GXEBOLA-CE-50
Cartridge box	5
Kit box	1
CD	Assay Definition File (ADF) Assay Import Instructions Package Insert (PDF)
Cartridges Per Kit	50 (5 boxes of 10)
Sample Reagent Bottles	50 x 2.5mL
Transfer Pipettes	50 x 1mL



Cartridges contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.

Xpert[®] Ebola* Kit Storage and Handling

- Store the Xpert Ebola Assay cartridges and reagents at 2–28°C
- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use Collection Reagent tubes that have not been validated by Cepheid
- Open the Assay cartridge lid only when adding the Sample, close the lid and proceed with processing



Warnings and Precautions

- Do not shake the cartridge
- Do not use a cartridge that... :
 - appears wet, has leaked or if the lid seal appears to have been broken
 - appears damaged
 - has been dropped after removing it from packaging
 - has been dropped or shaken after adding the sample to it
 - has a damaged reaction tube
 - has been used: each cartridge is single-use to process one test
 - is expired
 - Do not reuse spent disposable pipettes



Warnings and Precautions

- 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.

Xpert[®] Ebola* Assay limitations

- **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 and buccal swab 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 and buccal swab from individuals with Ebola Zaire virus infection.
- 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.

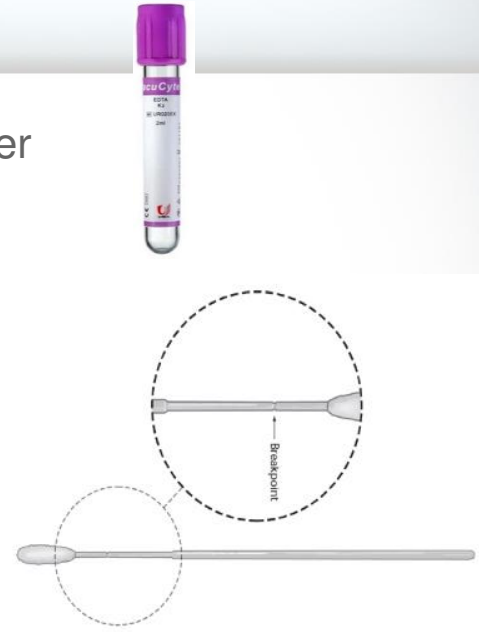
Specimen Collection, Storage and Transport



Whole Venous Blood sample collection

- Collect whole blood specimens by venipuncture in EDTA tubes per manufacturer's instructions
- Minimum of 100 μ L is required
- Have a swab* ready to be able to proceed with the specimen preparation

Swab - Cepheid catalog # SWAB/E-50 for CE-IVD kits*



Important:

Immediately proceed with the sample preparation step to ensure that the Ebola virus becomes inactivated

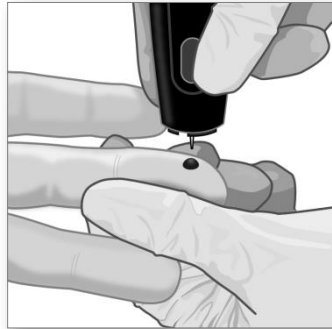
**WHO guidelines on how to safely collect blood samples*

<http://www.who.int/csr/resources/publications/ebola/blood-collect-en.pdf>

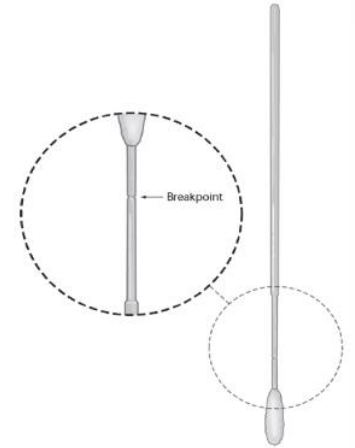
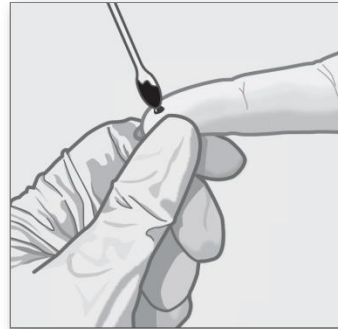
Capillary Blood sample collection from finger-stick

- Use the swab* to collect blood specimens from finger-stick
- Allow at least 2/3 of swab head to absorb blood

Swab* - Cepheid catalog # SWAB/E-50 for CE-IVD kits



Lancet
puncture

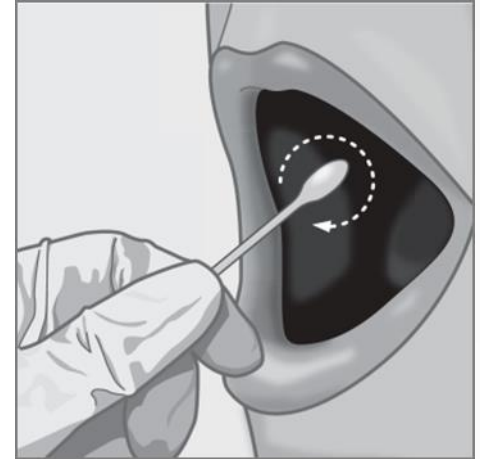


Important:


Immediately proceed with the sample preparation step to ensure that the Ebola virus becomes inactivated

Buccal sample collection

- Use the SWAB/E-50 to collect specimens
- immediately bring the swab tip to the inside of the cheek
- Avoid touching any other surface with the swab
- Swabs can be collected from living or deceased persons
- Collect the specimen according to WHO guidelines*
**WHO/EVD/Guidance/Lab/14.2*

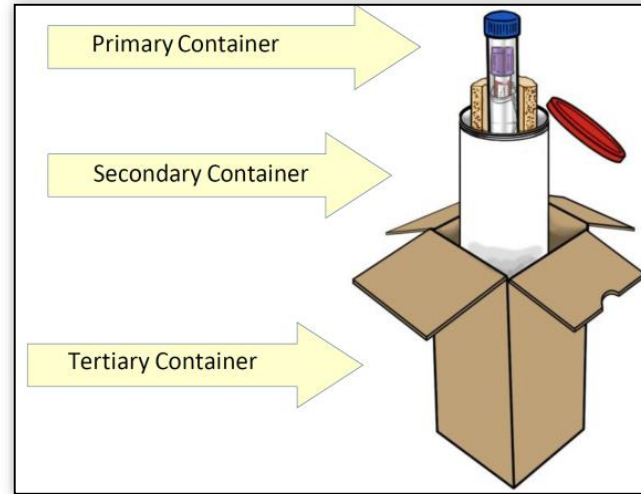


Specimen Storage

Temperature	 Sample type	Storage time
2-8°C	Reagent treated sample (blood or buccal)	72 hours
8-28°C	Reagent treated buccal sample	24 hours
	Reagent treated blood sample	48 hours
28-35°C	Reagent treated blood sample	24 hours

Specimen Transportation

- Transport Sample Reagent-treated samples to the testing laboratories according to WHO guidelines for transport of Ebola specimens



<http://www.who.int/csr/resources/publications/ebola/blood-collect-en.pdf>

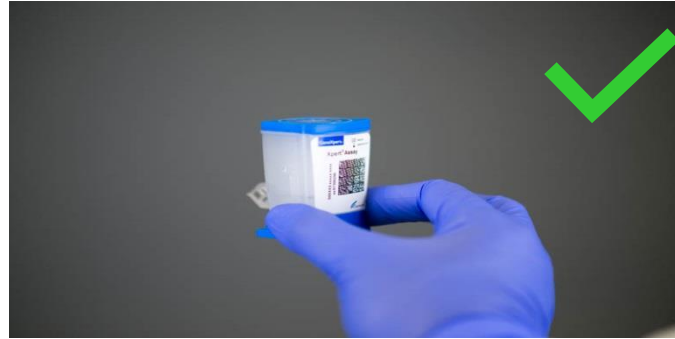
Cartridge Preparation



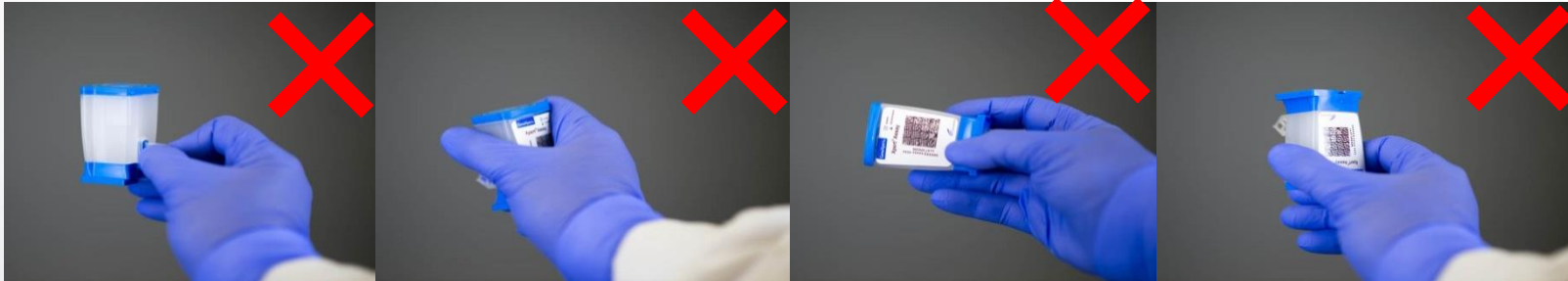
Proper Cartridge Handling Techniques

Correct

- Do not touch the reaction tube
- Keep the cartridge upright
- Do not tilt after sample is added



Incorrect



Xpert Ebola Cartridge Preparation

Whole Venous Blood in EDTA-tubes

1



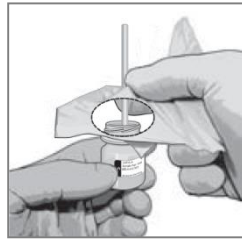
Label the Sample Reagent (SR) bottle with sample ID and open the lid

2



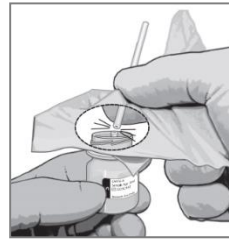
Place the swab in the EDTA tube. Allow to absorb for 5 seconds

3



Insert the swab into the SR bottle. Align the small groove against the edge (rim) of the bottle

4



Break off the swab by bending to one side

5



Close the lid of the SR bottle

6



Vortex the sample for 10 seconds

7



Incubate at room temperature for 20 minutes

8



Label the cartridge side with the same ID as the SR bottle. Open the cartridge lid

9



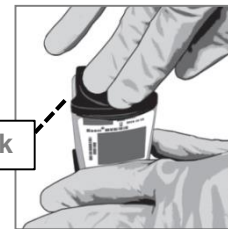
Aspirate at least 1mL of the sample in the pipette

10



Slowly empty the pipette into the sample chamber of the cartridge

11



Close the lid firmly

12

Max. 30min.



Start the test on GeneXpert

Xpert Ebola Cartridge Preparation

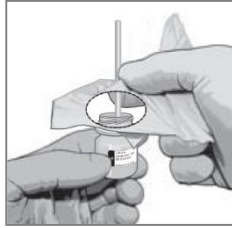
Capillary Blood swab from Finger-Prick or Buccal Swab

1



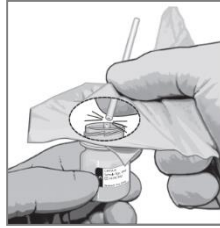
Label the Sample Reagent (SR) bottle with sample ID and open the lid

2



Insert the swab into the SR bottle. Align the small groove against the edge (rim) of the bottle

3



Break off the swab by bending to one side

4



Close the lid of the SR bottle

5



Vortex sample for 10 seconds

6



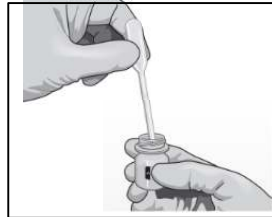
Incubate at room temperature for 20 minutes

7



Label the cartridge side with the same ID as the SR bottle. Open the cartridge lid

8



Aspirate at least 1mL of the sample in the pipette

9



Slowly empty the pipette into the sample chamber of the cartridge

10



Close the lid firmly

11

Max. 30 min.
-->



Start the test on GeneXpert

Run a Test

1 Create Test

GeneXpert



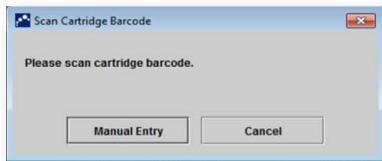
Start the test within **30 minutes** after adding the sample to the cartridge

GeneXpert
Infinity



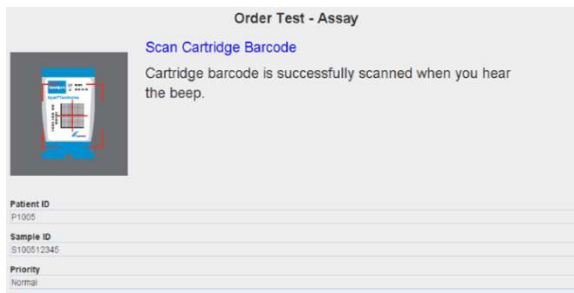
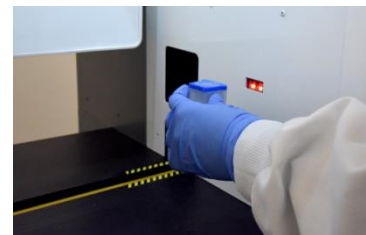
Place the cartridge on the conveyor within **30 minutes** of adding the sample.

2 Scan barcode messages: Cartridge/ Patient and/or Sample ID



*By default, do not click on
Manual Entry or **Cancel***

3 Scan the cartridge



"For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx or Xpertise Dx Operator Manuals.

Create a Test on GeneXpert Dx Software

4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 The module is selected automatically
DO NOT CHANGE IT!!!

7 Click on Start Test

8 A green light will flash on the module
Load the cartridge into module and close the door

Create Test

Patient ID

Sample ID

Patient ID 2

Last Name

Name

Select Assay Xpert® Ebola*

Select Module A3

Reagent Lot ID* 16119 Expiration Date* 2016/1/17

Test Type Specimen

Sample Type Other Other S

Notes

Start Test Scan Cartridge Barcode



Quality Controls



Cepheid Control Strategy

CONTROL

- **System Control – Check Status**

- System control checks the optics, temperature of the module and mechanical integrity of each cartridge.
- If the system controls fail, an ERROR test result will be reported.

- **Assay Quality Controls**

- Each Xpert cartridge is a self-contained test device
- Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge.
 - Probe Check Controls (PCC)
 - Cepheid Internal Control (CIC)
 - Sample Adequacy Control (SAC)

Internal Quality Controls

- **Probe Check Controls (PCC)**

- Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor
 - bead rehydration
 - reaction tube filling
 - probe integrity
 - dye stability

- **Cepheid Internal Control (CIC)**

- Armored RNA[®] included in each cartridge
 - Verifies adequate processing of the sample virus
 - Verifies lysis, presence of the organism and detects inhibition
 - Must be positive in a negative sample
 - Can be positive or negative in a positive sample

- **Sample Adequacy Control (SAC): HMBS (Hydroxymethylbilane synthase)**

- Ensures that human cells have been added in the sample chamber of the cartridge

Commercially Available External Controls

AccuPlex™ rEbola GP/NP Reference Material

Part Number	Description	Configuration	Storage
0505-0001	GP+NP positive control	250uL x 5 vials	2-8°C

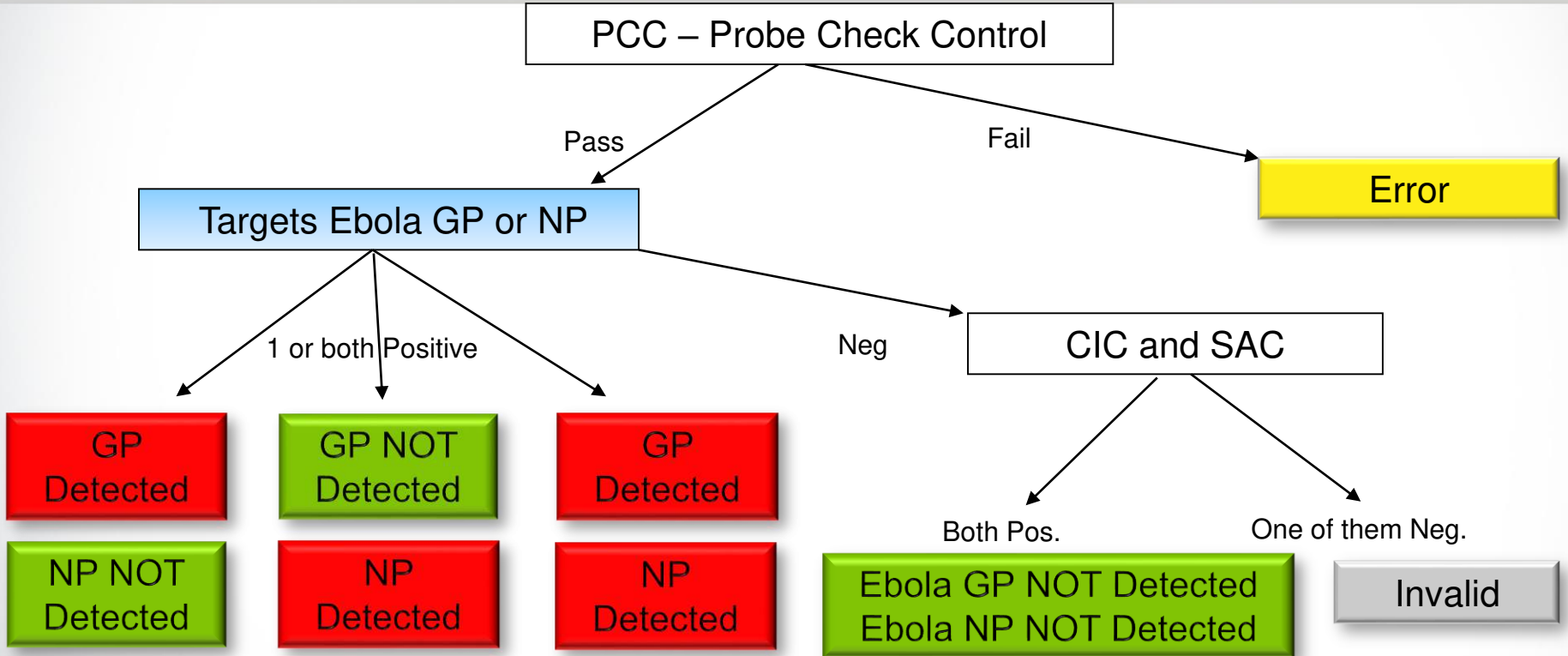
<http://www.seracare.com/>

- Other vendors for quality control material are also available than the one outlined above.
- External controls should be used in accordance with local, state accrediting organizations, as applicable

Result Interpretation

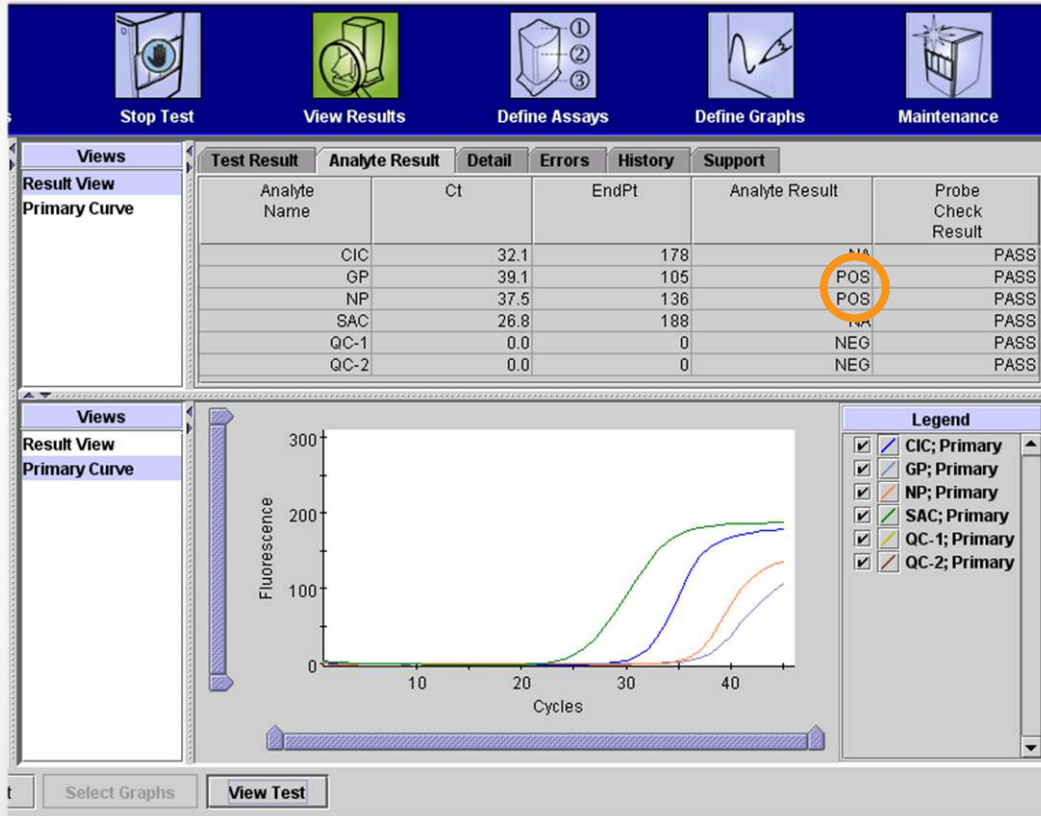


Result Interpretation Algorithm



Ebola GP DETECTED; Ebola NP DETECTED

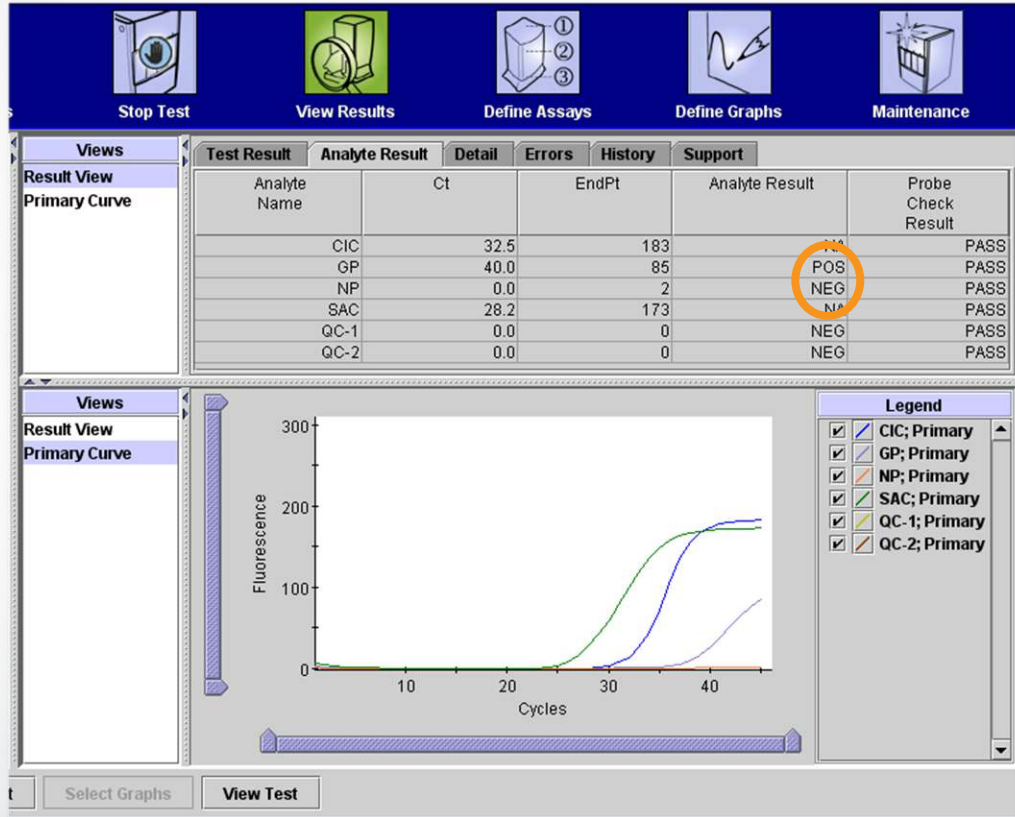
Test Result **Ebola GP DETECTED;
Ebola NP DETECTED**



- The EBOLA targets GP and NP are detected and the Ct values are within the valid range
- SAC: NA (not applicable)
 - SAC is ignored because the EBOLA target amplification occurred
- CIC: NA (not applicable)
 - CIC is ignored because the EBOLA target amplification occurred
- Probe Check: PASS

Ebola GP DETECTED; Ebola NP NOT DETECTED

Test Result **Ebola GP DETECTED;**
Ebola NP NOT DETECTED

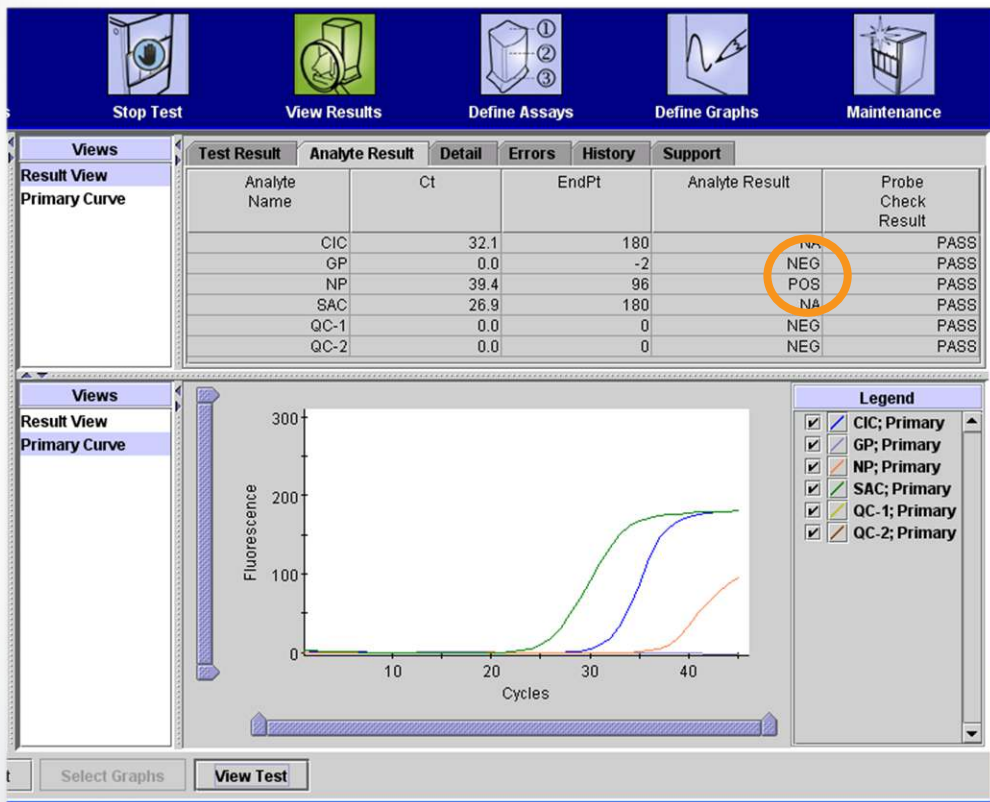


- The EBOLA target GP is detected and the Ct value is within the valid range
- SAC: NA (not applicable)
 - SAC is ignored because the EBOLA target amplification occurred
- CIC: NA (not applicable)
 - CIC is ignored because the EBOLA target amplification occurred
- Probe Check: PASS

Ebola GP NOT DETECTED; Ebola NP DETECTED

Test Result

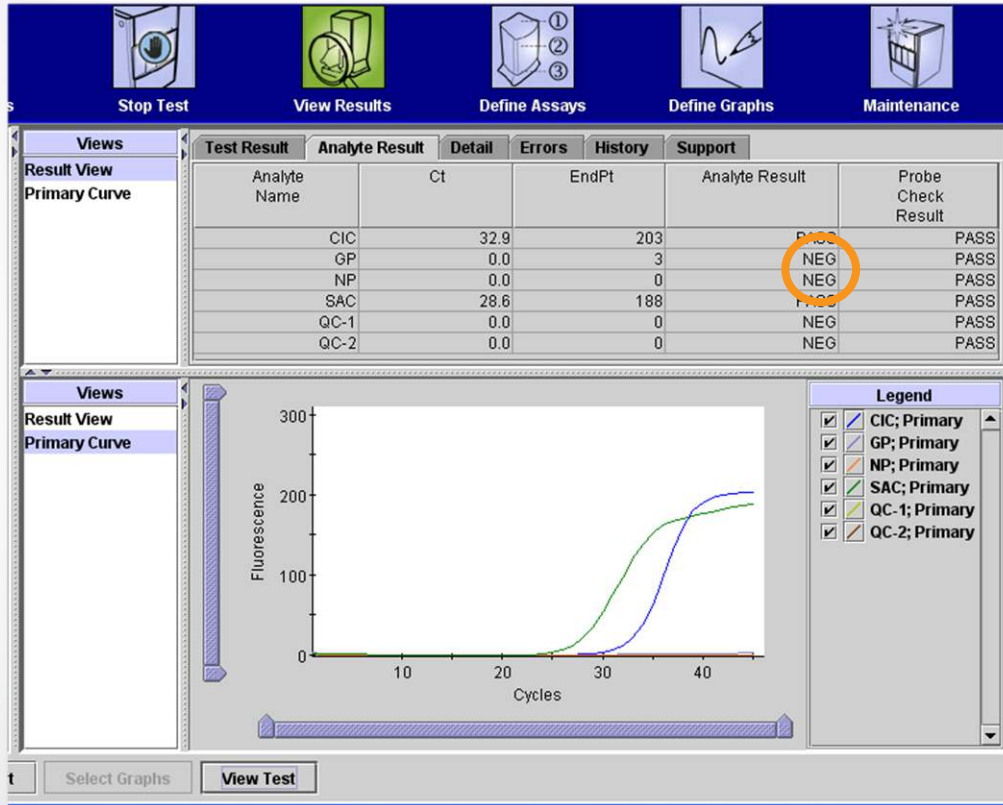
Ebola GP NOT DETECTED;
Ebola NP DETECTED



- The EBOLA target NP is detected and the Ct value is within the valid range
- SAC: NA (not applicable)
 - SAC is ignored because the EBOLA target amplification occurred
- CIC: NA (not applicable)
 - CIC is ignored because the EBOLA target amplification occurred
- Probe Check: PASS

Ebola GP NOT DETECTED; Ebola NP NOT DETECTED

Test Result **Ebola GP NOT DETECTED;
Ebola NP NOT DETECTED**



- The EBOLA targets GP and NP are NOT detected
- SAC: PASS
 - SAC has a Ct value within the valid range
- CIC: PASS
 - CIC has a Ct value within the valid range
- Probe Check: PASS

Troubleshooting

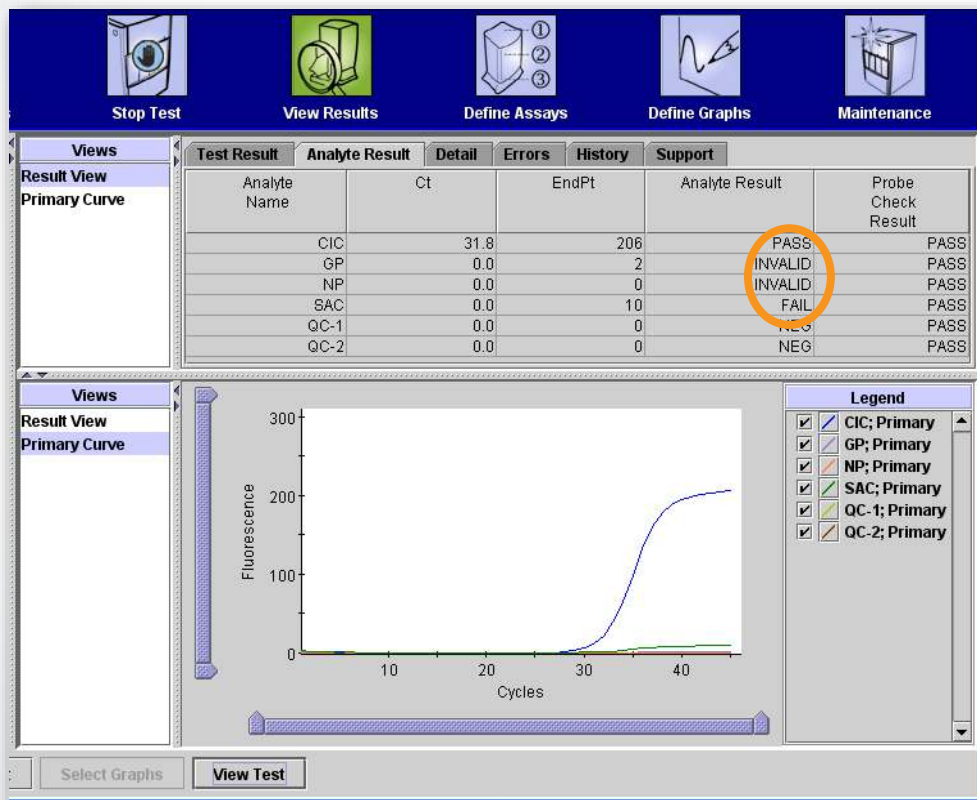


Factors That Negatively Affect Results

- Improper specimen collection
 - The viral load in the specimen is below the detection limit of the test
 - Performance with other specimen types has not been assessed
- Improper transport or storage of collected specimen
 - Storage and transport conditions are specimen specific
 - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
 - Modification to the testing procedures may alter the performance of the test
 - Careful compliance with the package insert is necessary to avoid erroneous results

INVALID Result

Test Result **INVALID**



. Presence or absence of the Ebola GP and NP targets cannot be determined.

- SAC: FAIL; signal does not have a Ct within valid range and/or endpoint below minimum setting
- Probe Check – PASS; all probe check results pass

Possible Causes

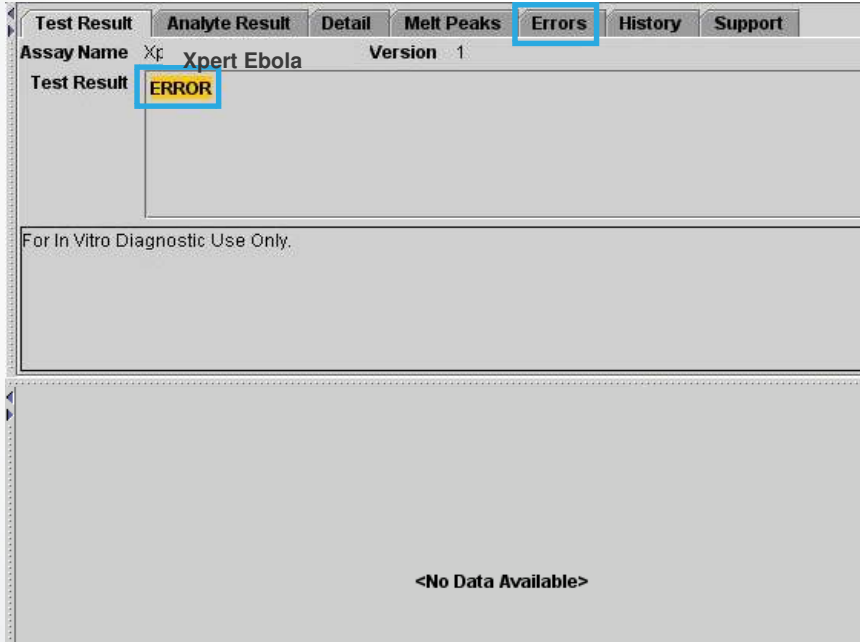
- Improper sample collection or preparation
- Presence of interfering substances in the sample

Solution

- Repeat the test with a new cartridge

ERROR

ERROR



Presence or absence of the NP or GP targets cannot be determined. Repeat test according to the Retest Procedure in IFU.

GP/NP: NO RESULT

SAC: NO RESULT

Probe Check: FAIL; all or one of the probe check results fail

If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.

Solution

Repeat the test with a new cartridge.

NO RESULT

NO RESULT

The screenshot shows the Xpert Ebola software interface. At the top, there are several tabs: 'Test Result', 'Analyte Result', 'Detail', 'Melt Peaks', 'Errors', 'History', 'Messages', and 'Support'. Below the tabs, the 'Assay Name' is 'Xpert Ebola' and the 'Version' is '1'. The 'Test Result' field is highlighted with a blue box and contains the text 'NO RESULT'. Below the 'Test Result' field, there is a section labeled 'For In Vitro Diagnostic Use Only.' which is currently empty.

Possible Causes

A NO RESULT indicates that insufficient data were collected.

- Test was stopped with stop test button
- Electrical failure

Solution

- Secure the power
- Repeat the test with a new cartridge.

Presence or absence of GP/NP cannot be determined.
A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.
GP/NP: NO RESULT
SAC: NO RESULT
Probe Check: NA (not applicable)

Re-test Procedure

1



Discard used cartridge

Follow your institution's safety guidelines for disposal of cartridges

2



Obtain the residual sample, mix according to Package Insert

If the leftover sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample

3



Obtain a new cartridge

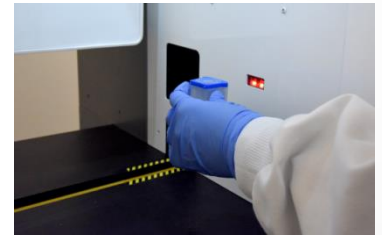
Label appropriately as retest on the new cartridge

Process the sample per the package insert

4



Run the test on the System



Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
 - Product name
 - Lot number
 - Serial number of the System
 - Error messages (if any)
 - Software version and, if applicable, Computer Service Tag number
- Log your complaint online using the following link <http://www.cepheid.com/us/support> :
Create a Support Case



Thank You.



www.Cepheid.com