Assay Technical Training
Xpert® HIV-1 Qual

For CE-IVD Use Only

Cepheid Training Center

CE IVD In Vitro Diagnostic Medical Device

301-4906 Rev. C May 2019

Training Agenda

- **Xpert HIV-1 Qual**
  - Clinical utility
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Preparing the cartridge
  - Quality Controls
  - Results analysis
- **Discussion**

Cepheid

Training Objectives

- **At the end of the training, user will be able to:**
  - Properly store and handle the Xpert® HIV-1 Qual cartridge kit and sample collection kits
  - Follow proper laboratory safety precautions
  - Collect appropriate specimen types and transport specimen
  - Perform the cartridge set up and run the assay
  - Understand assay control strategy
  - Report the various software generated results

The Cepheid Solution

- Detects one target
  - HIV-1
- On-board controls for each individual sample
  - Probe Check Control (PCC)
  - Specimen Processing Control (SPC)
  - Sample Volume Adequacy (SVA)
- Results in approximately 90 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

Intended Use

- **The Xpert HIV-1 Qual Assay, is a qualitative *in vitro* diagnostic test designed to detect Human Immunodeficiency Virus Type 1 (HIV-1) total nucleic acids using human whole blood (WB) and Dried Blood Spots (DBS) from individuals suspected of HIV-1 infection.**

- **The Xpert HIV-1 Qual Assay is intended to aid in the diagnosis of HIV-1 infection in conjunction with clinical presentation & other laboratory markers.**

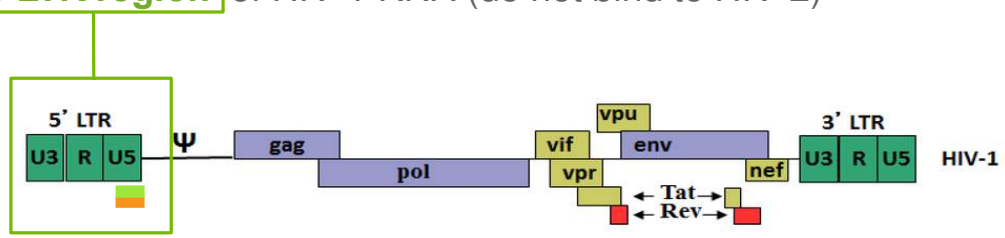
- **This assay is not intended to be used as a donor screening test for HIV-1 infection.**

- **This assay uses reverse transcription polymerase chain reaction (RT-PCR) technology.**

Targets and Probes

- **Target**
  - HIV-1

- **Probes**
  - 1 probe binds to the SPC
  - 2 probes bind to **3' end** of **5'LTR region** of HIV-1 RNA (do not bind to HIV-2)



  - **Depending on the HIV group, one of the probes will bind**.

http://www.mdpi.com/2227-9059/2/1/14/htm

Assay Requirements

GeneXpert Systems

- GeneXpert Dx Software **v4.7b** or higher
- Infinity Dx Software **v6.4b** or higher

Test Kits (CE-IVD)

- GXHIV-QA-CE-10

Sample Collection Whole Blood Protocol

- EDTA tubes

Sample Collection Dried Blood Spots Protocol

DBS Collection Kit *(Whatman 903 Paper card or equivalent)*
Sterile Scissors and/or forceps
Eppendorf Thermomixer® C *(Eppendorf reference 5382 000.015)*
Eppendorf Smartblock™ (vial rack) *(Eppendorf reference 5309 000.007)*

Other materials

- Personal Protective Equipment (PPE)
- 1:10 Bleach and 70% ethanol or denatured ethanol

Optional

- Uninterruptible Power Supply /Surge Protector
- Printer

Good Laboratory Practice

Personnel Protective Equipment (PPE)	• Wear clean lab coats and gloves • Change gloves between processing samples
Lab Bench area	• Clean work surfaces routinely with: ✓ 1:10 dilution of household bleach ✓ 70% Ethanol Solution • 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 tips when recommended • Follow the manufacturer's requirements for calibration and maintenance of equipment(s)

Kit Handling

Xpert HIV-1 Qual Kit Contents

Catalog Number	GXHIV-QA-CE-10
Tests Per Kit	10
Other reagent	Sample Reagent
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Transfer Pipettes	10 (1mL) and 10 (100uL)
Storage	2-28 ˚C

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

Xpert HIV-1 Kit Storage and Handling

- Store the Xpert HIV-1 Qual 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
- With GeneXpert System, start the test within 30 minutes after adding the sample to the cartridge
- With the Infinity System, place the cartridge on the conveyor within 30 minutes after adding the sample.

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

Dispose Xpert HIV-1 Qual Assay cartridges and reagents according to your institution's and country's guidelines for disposal of hazardous materials

Xpert HIV-1 Qual Limitations

- The performance of Xpert HIV-1 Qual has not been demonstrated from specimens other than the ones validated, i.e. Whole Blood and Dried Blood Spots
- The performance of the Xpert HIV-1 Qual test has not been evaluated with specimens processed by methods other than those described in the package insert.
- Failure to follow assay procedures may lead to false negative results.
- Inhibitors present in the samples may lead to invalid results.

For detailed information, refer to the current Package Insert

Specimen Collection, Storage and Transport

Whole Blood Collection

- Collect whole blood specimens in EDTA tubes as per manufacturer's instructions
- After blood collection, mix the specimen by gently inverting the tube **10 times**
- A minimum of 100µL is required for testing

EDTA tube

Heparin tube

Cepheid

Dried Blood Spot (DBS) Collection

- Collect dried blood spot (DBS) specimen
- DBS should be prepared using Whatman 903, Munktell filter paper cards or equivalent from blood obtained from:
  - a heel-stick
  - finger-stick
  - toe-stick
  - an EDTA-tube
- Ensure that the entire circle is covered with blood (approximately 60–70 µL). A minimum of two circles should be made from each sample to allow for retesting.
- Air-dry the card at room temperature for a minimum of 4 hours. Package each card in an individual resealable bag with a desiccant sachet in each bag.

https://www.spotonsciences.com/knowledge-center/dbs-technology/

For further details about DBS preparation and packaging please refer to related chapter in "WHO DBS drug resistance testing Manual"

Specimen Collection, Transport and Storage

	Prior to testing	Temperature (°C)	Storage Time
	**EDTA anticoagulated whole blood**	+31 +35 °C	8 hours
		+15 +25 °C	24 hours
		+2 +8 °C	72 hours

Sent to the testing laboratories in individual re-sealable bags containing desiccant

	Prior to testing	Temperature (°C)	Storage Time
	**Dried Blood Spots (DBS)**	+2 +25 °C -15 °C	12 weeks
		+31 +35 °C	8 weeks

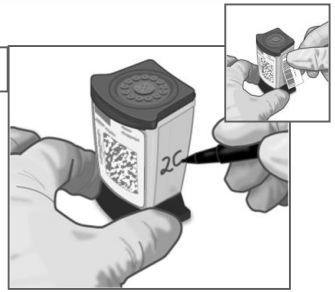
Cartridge Preparation

Cartridge Preparation - Whole Blood

1

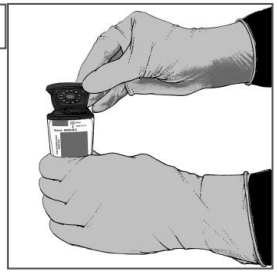
Label the Sample Reagent (SR) bottle with Sample ID

2

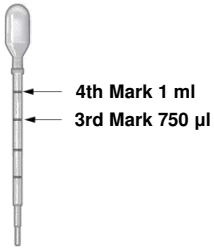


Label the side of the cartridge with the same ID as the SR bottle

3

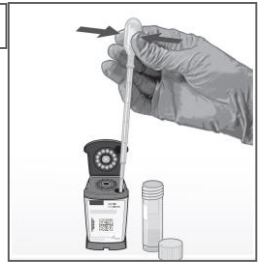


Open the cartridge lid



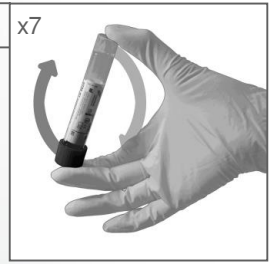
**HIV-1 Qual Assay 1 mL Transfer pipette*

4

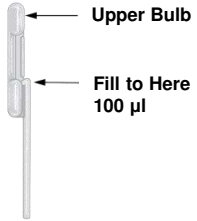


Use the transfer pipette* provided (or an automatic pipette) to transfer 750 µL of the sample reagent into the sample chamber of the cartridge

5 x7



Mix the whole blood sample by inverting the vial at least seven times



***HIV-1 Qual Assay 100 µL Transfer Micropipette*

6

Immediately transfer 100 µL of whole blood using the Micropipette** provided (or an automatic pipette) into the same sample chamber

7

Close the lid firmly

Start the test within the time frame specified in the package insert.

Adapted from 301-5060

Cepheid

Cartridge Preparation – Dried Blood Spots

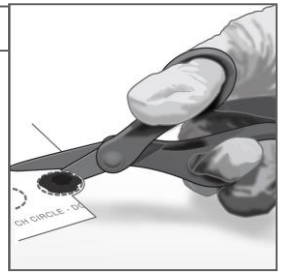
1

Set the Thermomixer C to 56⁰C

2

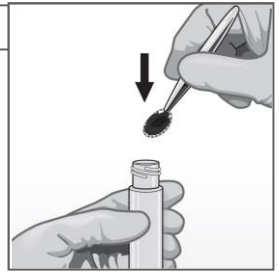
Label the Sample Reagent (SR) bottle with Sample ID

3



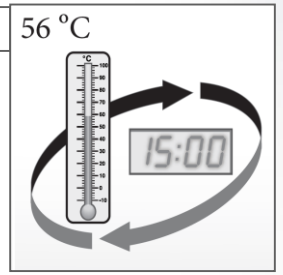
Using sterilized scissors, cut out one DBS from the filter paper card following the dashed line. If the dashed line is perforated, use forceps to detach the DBS

4



Place one DBS into the SR vial and make sure it is submerged in the buffer

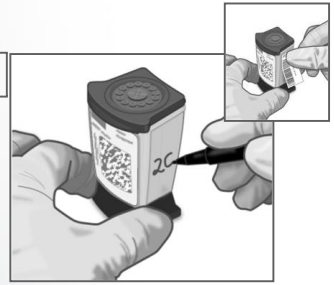
5



Incubate this vial in the ThemoMixer C for

- **15 minutes at**
- **56⁰C at 500 rpm**

6



Label the side of the cartridge with the same ID as the SR bottle

7

Open the cartridge lid

**HIV-1 Qual Assay*
1 mL Transfer pipette

Adapted from 301-5060

8

Use the provided transfer pipette* (or an automatic pipette) to transfer all the liquid from the vial into the sample chamber of the cartridge

9

Close the lid firmly

Start the test within the time frame specified in the package insert.

Run a Test

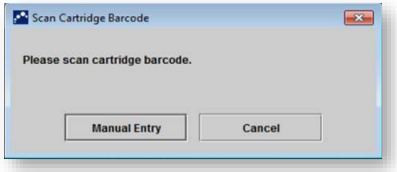
1 Create Test

GeneXpert

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

2 Scan barcode messages:

Cartridge/ Patient and/or Sample ID

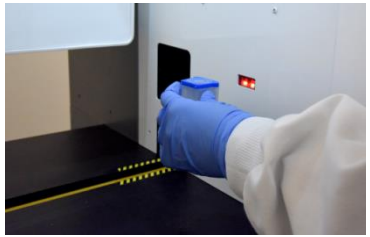


By default, do not click on **Manual Entry** or **Cancel**

3 Scan the cartridge

GeneXpert Infinity

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



"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

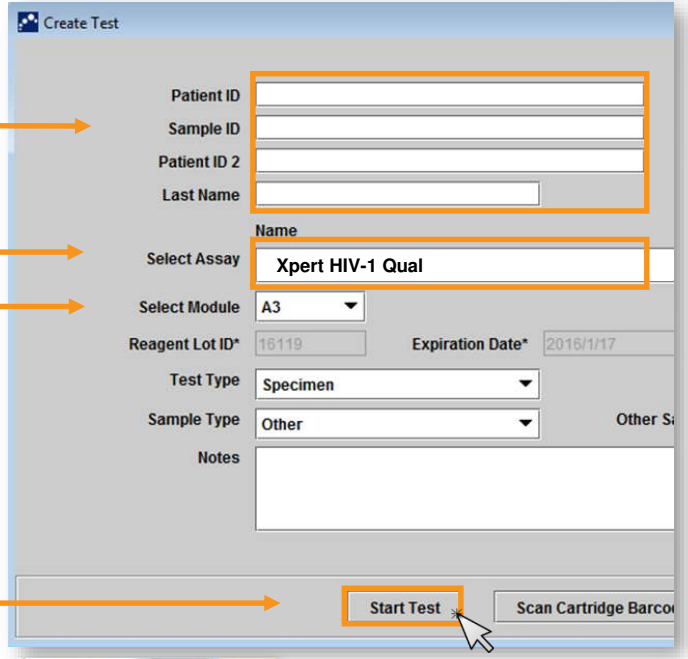
4 Complete the fields as required

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

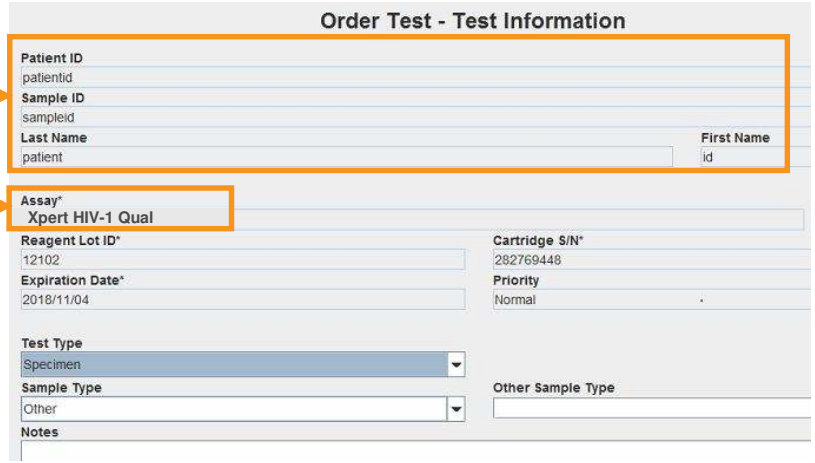


Create a Test on Xpertise Dx Software

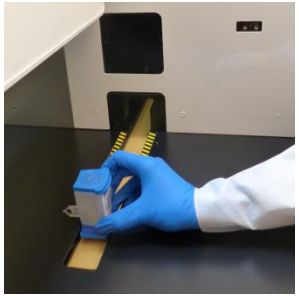
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 Click on SUBMIT



7 Place the cartridge onto the conveyor belt



Cepheid

Automated Xpert Protocol

1. Sample is added to the cartridge
2. The cartridge is loaded into the System
3. Nucleic acids are purified
4. Purified nucleic acids mix with the PCR reagents
5. Simultaneous amplification and detection occurs
6. Results are ready to view

Cepheid

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
    - Sample Volume Adequacy (SVA)
    - Specimen Processing Control (SPC)
    - Probe Check Controls (PCC)

Internal Quality Controls

- **Sample Volume Adequacy (SVA)**
  - Verifies that the correct sample volume is added to the cartridge
- **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
- **Sample Processing Controls (SPC)**
  - Armored RNA
    - Verifies adequate sample processing
    - Verifies lysis and presence of the organism and detects PCR inhibition
    - Should be positive in a negative sample
    - Can be positive or negative in a positive sample

Cepheid

Commercially Available External Controls

AcroMetrix® http://www.lifetechnologies.com/acrometrix

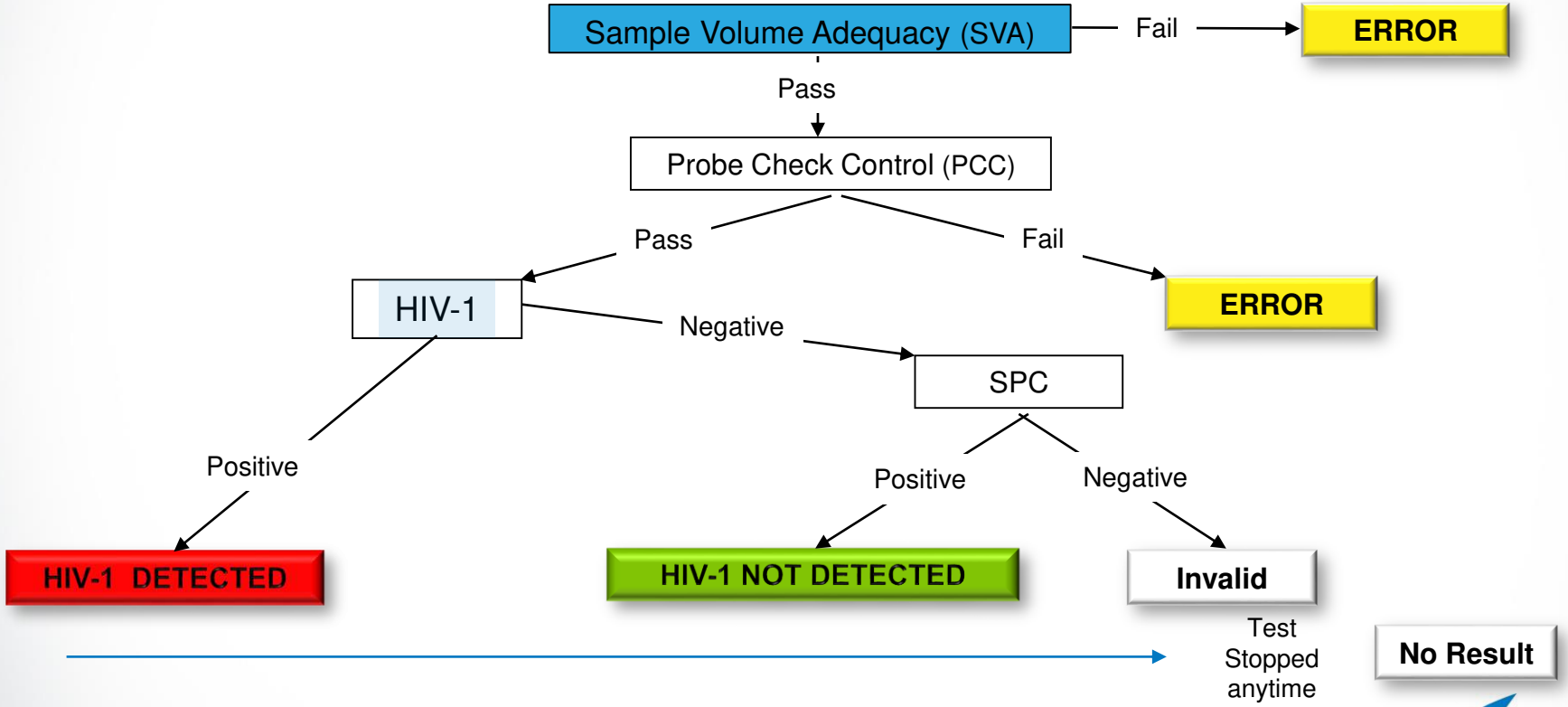
Part Number	Description	Configuration	Storage
964003	HIV-1 High control	1.2 mL x 5 vials	≤ -20°C
964002	HIV-1 Mid control	1.2 mL x 5 vials	≤ -70°C
964001	HIV-1 Low control	1.2 mL x 5 vials	≤ -70°C

1. *Take 1 vial of the control material*
2. *Thaw it at ambient temperature and mix well*
3. *Immediately after thawing place the vial in the ice*
4. *Transfer the entire amount (1.2mL) using the transfer pipette of the Xpert HIV-1 kit and add it to the Xpert HIV-1 sample chamber of the cartridge*
5. *Close the lid and launch the test on GeneXpert*

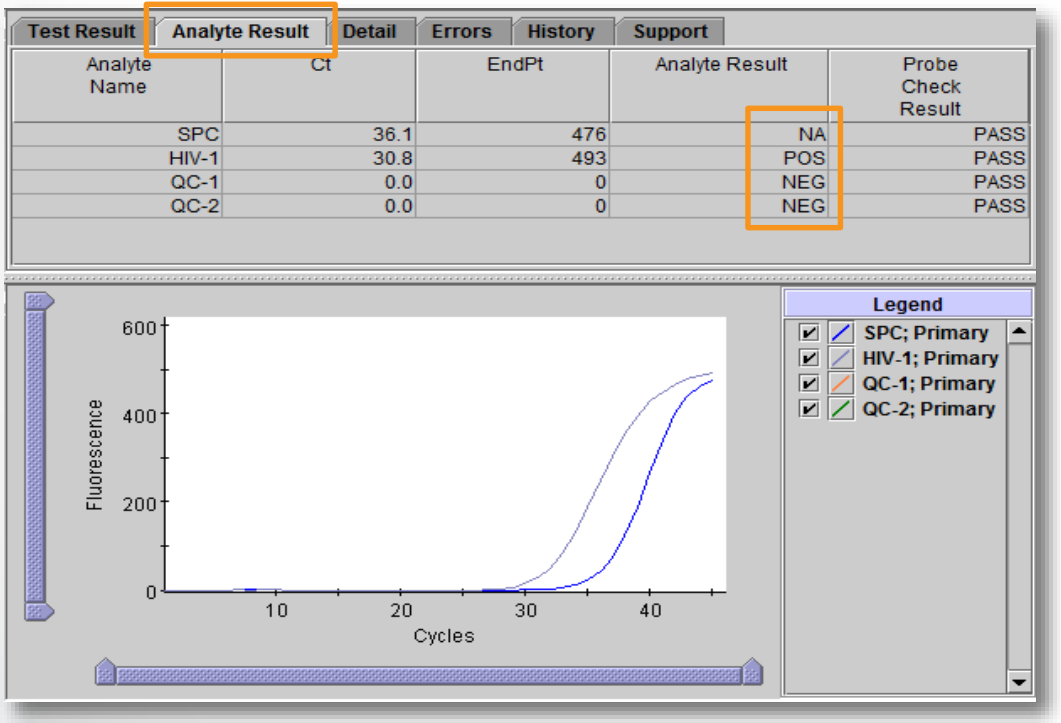
- Many other vendors for quality control material are also available than the one outlined above.
- External controls should be used in accordance with local, state accrediting organizations, as applicable

Result Interpretation

Result Interpretation Algorithm



HIV-1 DETECTED



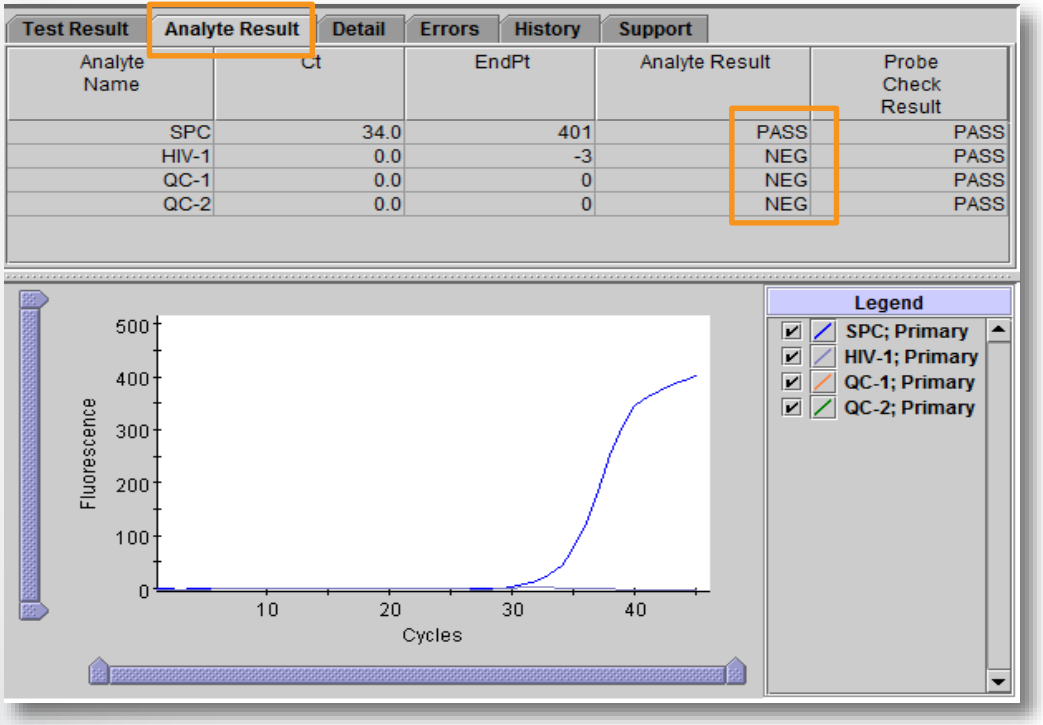
The target HIV-1 is detected

- SPC: NA (Not Applicable) SPC is ignored when HIV-1 target amplified
- Probe Check: PASS

Cepheid

HIV-1 NOT DETECTED

HIV-1 NOT DETECTED



The target HIV-1 is NOT detected

- SPC: PASS

SPC has a Ct value within the valid range

- Probe Check: PASS

Cepheid

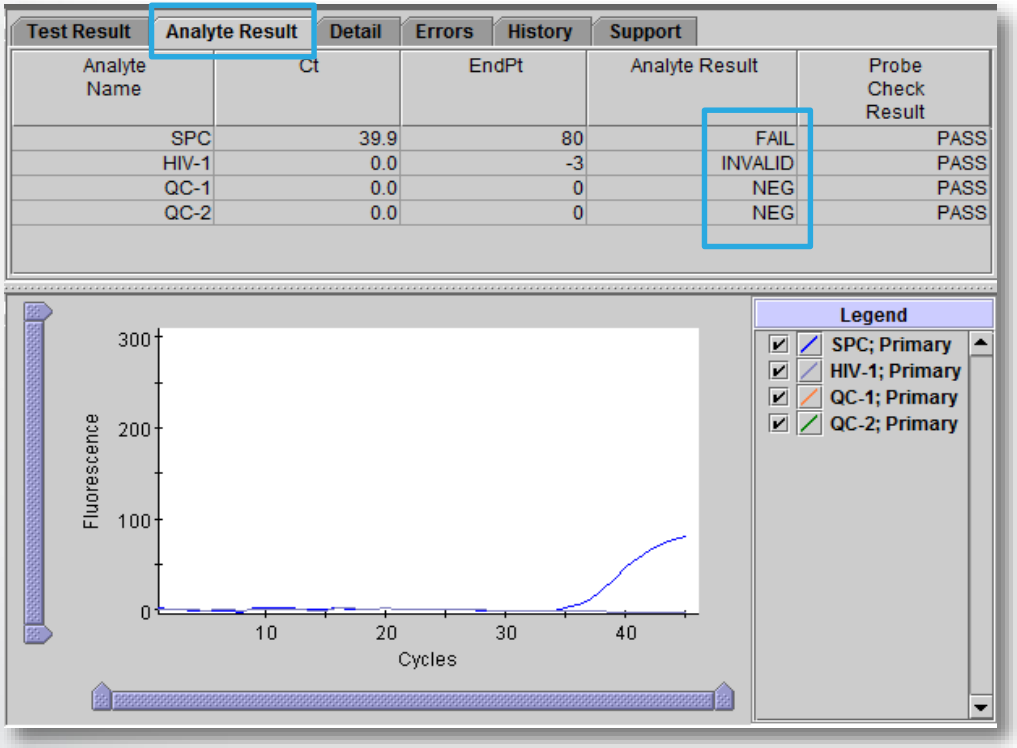
Troubleshooting

Factors That Negatively Affect Results

- Improper specimen collection
  - Performance with other collection devices and specimen types has not been assessed
- Improper transport or storage of collected specimen
  - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
  - Modification to the testing procedures, technical error and sample mix-up may impact the test results
  - Careful compliance with the package insert is necessary to avoid erroneous results
- Interfering substance
  - False negative results or invalid results may be observed in the presence of interfering substance

INVALID Result

INVALID



Presence or absence of the HIV-1 target can not be determined

- SPC: FAIL
  - SPC Ct value is not within the valid range
- Probe Check: PASS

– Possible Causes
  – Improper sample collection (using heparin tube for e.g)
  – Incorrect sample preparation
  – Improper storage of the cartridges
  – Inefficient sample processing in cartridge
  – Presence of inhibitors in the sample

– Solution
  – Repeat the test with a new cartridge and new sample

Assay Interference

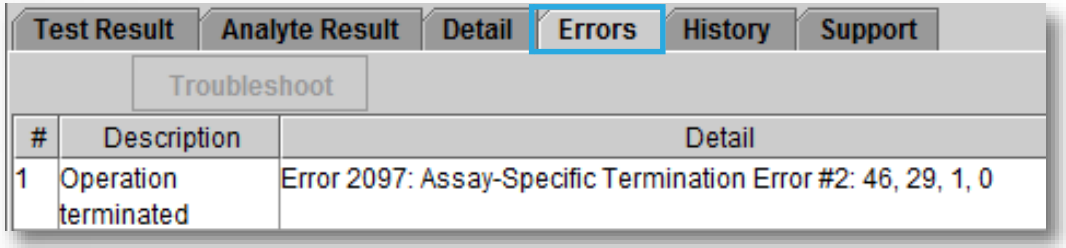
- Potentially Interfering Substances
  - A total of 5 endogenous substances were evaluated
  - Elevated levels of those endogenous substances were shown not to impact the assay specificity or interfere with the HIV-1 detection

Substance	Tested Concentration
Albumin (BSA)	90 mg/mL
Bilirubin	0.2 mg/mL
Hemoglobin	5 mg/mL
Human DNA	4 µg/mL
Triglycerides	30 mg/mL

For detailed information, refer to the current Package Insert

ERROR Result (2096/2097)

ERROR

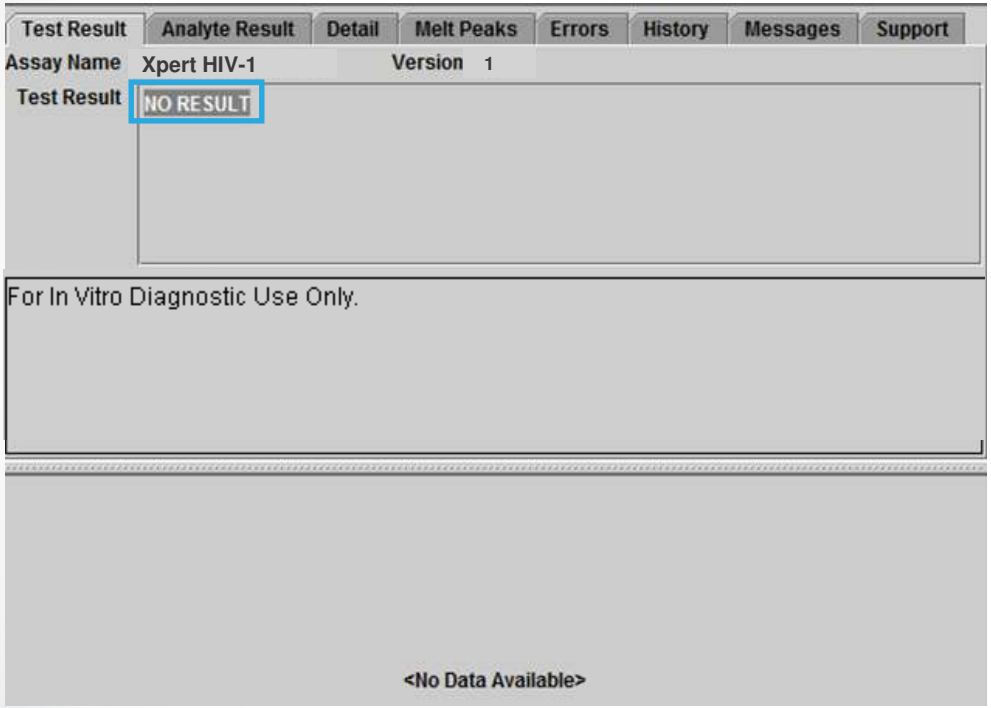


The Sample Volume Adequacy (SVA) passes if it meets the validated acceptance criteria.

Error Code	Cause	Solution
2096	No sample added	– Ensure the Sample is added to cartridge – Ensure cartridge is loaded within 30 min. after adding sample
2097	Not enough sample added	– Ensure the minimum sample volume is added to the cartridge – Ensure cartridge is loaded within 30 minutes after adding sample

Cepheid

NO RESULT



- The presence or absence of HIV-1 cannot be determined.
  - TARGET 1: NO RESULT
  - CONTROL: NO RESULT
- Probe Check: NA (not applicable)

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

Cepheid

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

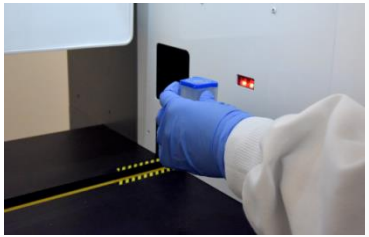
Obtain a new cartridge

Label appropriately as retest on the new cartridge

Process the sample per the package insert

4

Run the test on the GeneXpert System



Cepheid

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 you complaint on the following link ***http://www.cepheid.com/us/support***

Region	Telephone	Technical Support Email
US	+ 1 888 838 3222	techsupport@cepheid.com
Australia and New Zealand	+ 1800 130 821 + 0800 001 028	techsupportanz@cepheid.com
Brazil and Latin America	+ 55 11 3524 8373	latamsupport@cepheid.com
China	+ 86 400 821 0728	techsupportchina@cepheid.com
Japan	0120 95 4886	support@japan.cepheid.com
France	+ 33 563 825 319	support@cepheideurope.com
Germany	+ 49 69 710 480 480	support@cepheideurope.com
India, Bangladesh, Bhutan, Nepal and Sri Lanka	+ 91 11 48353010	techsupportindia@cepheid.com
Italy	+ 39 800 902 567	support@cepheideurope.com
South Africa	+ 27 861 22 76 35	support@cepheideurope.com
United Kingdom	+ 44 3303 332 533	support@cepheideurope.com
Other European, Middle East, and African countries	+ 33 563 825 319 + 971 4 253 3218	support@cepheideurope.com
Other countries not listed above	+1 408 400 8495	techsupport@cepheid.com


Thank You.
www.Cepheid.com
GeneXpert
Xpert
Cepheid
Cepheid