Technical Training
Xpert® HIV-1 Viral Load

Catalog Number (GXHIV-VL-CE-10)
For CE-IVD Only

CE 2797 IVD In Vitro Diagnostic Medical Device

301-4648 Rev. E January 2024

Training Agenda

1. Reagents
2. Sample collection
3. Kit storage and handling
4. Preparing the cartridge
5. Quality controls
6. Results analysis
7. Discussion

Training Objectives

At the end of the training, users will be able to:

- Properly store and handle the Xpert® HIV-1 Viral Load cartridge kit and sample collection
- Follow proper laboratory safety precautions
- Collect and transport appropriate specimen
- Prepare a cartridge and run the Xpert® HIV-1 Viral Load test
- Report the various software generated results
- Understand the Xpert® HIV-1 Viral Load control strategy

The Cepheid Solution

- Detects and quantifies HIV-1 target
  - Reliable results with a linear range from 40 - 10,000,000 HIV-1 RNA copies/mL
- On-board internal controls for each sample
  - Sample Volume Adequacy (SVA)
  - Probe Check Control (PCC)
  - Internal Quantitative Standards (IQS) High (H) and Low (L)
- Results in 90 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

Intended Use

- The Xpert® HIV-1 VL test is an in vitro reverse transcriptase polymerase chain reaction (RT-PCR) test for the detection and quantification of Human Immunodeficiency Virus type 1 (HIV-1) RNA in human plasma from confirmed HIV-1 positive adults with a known antiviral treatment status, using the automated GeneXpert Instrument Systems.
- The test can quantify HIV-1 RNA over the range of **40 to 10,000,000 copies/mL**. The Xpert HIV-1 VL test is validated for quantification of RNA from HIV-1 **Group M** (subtypes A, B, C, D, F, G, H, J, K, CRF01_AE, CRF02_AG, and CRF03_AB), **Group N**, and **Group O.**
- The Xpert ® HIV-1 VL test is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels.

Intended Use continued

- The test is intended to be used by **laboratory professionals** or **specifically-trained healthcare workers.**
- The Xpert® HIV-1 VL test is not intended to be used as a donor screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.
- The intended patient population for the Xpert® HIV-1 VL test is confirmed HIV-1 positive adults, with a known antiviral treatment status.

Specimen, Targets and Probes

- **Specimen**
  - Human Plasma collected with K2 EDTA or EDTA (PPT) or ACD tubes
- **Target**
  - **HIV-1**
- **Probes**
  - 1 probe binds to IQS-H
  - 1 probe binds to IQS-L
  - 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

Xpert® HIV-1 Viral Load Requirements

GeneXpert® Systems

- GeneXpert Dx software **v4.7b** or higher
- Xpertise software **v6.4b** or higher
- GeneXpert Edge Software **v1.0** or higher

Test Kits

- Catalog Number (GXHIV-VL-CE-10)

Sample Collection

- K2 EDTA / EDTA-PPT or ACD tubes

Other Materials

- Personal Protective Equipment (PPE)
- 10% Bleach / Sodium Hypochlorite
- 70% ethanol or denatured ethanol
- Vortex
- Centrifuge for plasma preparation

Other Materials

- Uninterruptible Power Supply /Surge Protector
- Printer

Good Laboratory Practice Review

Personal Protective Equipment (PPE)	• Wear clean lab coats, safety glasses, 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 samples away from kit to prevent contamination
Equipment	• Follow the manufacturer's requirements for calibration and maintenance of equipment

* Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country.

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Kit Handling

Xpert® HIV-1 Viral Load Kit Contents

Catalog Number	GXHIV-VL-CE-10
Cartridges* Per Kit	10
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Transfer Pipettes	10 (1ml)
Storage	2-28 °C

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

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[16] and the Clinical and Laboratory Standards Institute.[17]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Consult your institution’s environmental waste personnel on proper disposal of used cartridges and unused reagents. Check state, territorial, or local regulations as they may differ from national disposal regulations. This material may exhibit characteristics of hazardous waste requiring specific disposal requirements. Institutions should check their country hazardous waste disposal requirements.

16. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. Richmond JY and McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.
17. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition). .

Warnings and Precautions

- Do not substitute Xpert® HIV-1 VL test reagents with other reagents.
- Do not open the Xpert® HIV-1 VL test cartridge lid until you are ready to add the plasma specimen.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label.
- Each single-use Xpert® HIV-1 VL test cartridge is used to process one specimen. Do not reuse spent cartridges.
- Do not use a cartridge that has a damaged reaction tube
- Single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge that has been dropped after removing it from the packaging.

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

Warnings and Precautions continued

- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a solution of 1:10 dilution of household chlorine bleach and then 70% ethanol. Wipe work surfaces dry completely before proceeding.

- 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.
- For Instrument System cleaning and disinfecting instructions, refer to the appropriate *GeneXpert® Dx System Operator Manual*, *GeneXpert® Infinity System Operator Manual, or GeneXpert ® Edge System User's Guide*

Test Limitations

- The test targets a single conserved part of the LTR region with a combination of several oligonucleotides designed to accommodate polymorphisms in the genome. Rare mutations, base changes, deletions or inserts, within the LTR region of the Xpert® HIV-1 VL test may affect primer and/or probe binding resulting in under-quantification or lack of detection of virus. Users are advised to consider these events when evaluating HIV-1 viral load results; Xpert® HIV-1 VL results indicating viral suppression may require further testing using alternative technologies with different genomic targets in circumstances where poor medication adherence, accompanying laboratory data or other clinical information raise concerns of underlying viremia. The laboratory is also advised to perform method correlation studies if HIV testing methods change from one technology to another as differences between platforms and technologies may result in variable HIV viral load results.
- The Xpert® HIV-1 VL test has been validated only for use with K2 EDTA (including PPT-EDTA) and ACD plasma. Using this test to analyze other types of samples may give inaccurate results.

Test Limitations continued

- A negative test result does not preclude HIV-1 infection. Therefore, this test should not be used as a diagnostic test to confirm the presence of HIV-1 infection.
- Patients who have received CAR-T therapies may display positive results with Xpert® (HIV-1 Qual XC, HIV-1 VL, etc.) as the result of the presence of the LTR target within certain chimeric antigen receptor T-cell (CAR-T) products. Additional confirmatory testing should be performed to determine the patient's HIV status in people who have received

  CAR-T treatment.

Specimen Collection, Storage and Transport

Specimen Collection

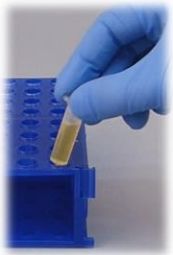
- **Whole blood**
  - Collect whole blood specimens in K2 EDTA, EDTA-PPT or ACD tubes as per manufacturer's instructions

K2 EDTA tube

BD® Vacutainer tube

Heparin tube

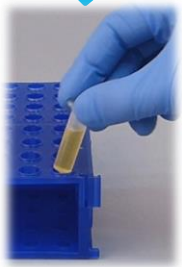
- **Plasma**
  - Centrifuge to separate the plasma and red blood cells per the manufacturer's instructions
  - Prepare minimum of 1.2mL of plasma



Specimen Collection, Transport and Storage

	Prior to testing	Temperature (°C)	Storage Time
	**K2 EDTA anticoagulated whole blood**	+15 to +25 °C	24 hours
		+2 to +8 °C	72 hours

Plasma specimens are stable up to three freeze/thaw cycles.

	Prior to testing	Temperature (°C)	Storage Time
	**Plasma**	+15 to +30 °C	24 hours
		+2 to +8 °C	6 days
		-70 to -18 °C	6 weeks

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

Cartridge Preparation Card

Xpert® HIV-1 VL Cartridge Preparation

- Xpert® HIV-1 Viral Load

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit
www.cepheid.com or
www.cepheidinternational.com
Cepheid Technical Support

US office
(888) 838-3222, Option 2
techsupport@cepheid.com

European office
+33 563 825 319
support@cepheideurope.com

Prior to beginning this test:

1. Centrifuge the sample to separate the plasma and red blood cells per the manufacturer's instruction.
2. Equilibrate the plasma to room temperature (20-35 ºC).
3. Vortex plasma for 15 seconds. If the specimen is cloudy, clarify with a quick spin.

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A better way.

1 Take one Xpert cartridge for each sample.

2 Open the cartidge lid.

3 Aspirate the plasma to just above the 1mL mark on the pipette.

4 Empty the contents into the sample chamber.

5 Close the cartridge lid.

6 Start the test within the timeframe specified in the package insert.

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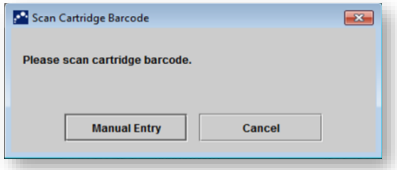
Run a Test on GeneXpert® Dx

1 Create a test.

Start the test within **4 hours** after adding the sample to the cartridge.

2 Scan barcode for Patient and/or Sample ID.



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 Operator Manual.

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Run a Test on GeneXpert® Dx (continued)

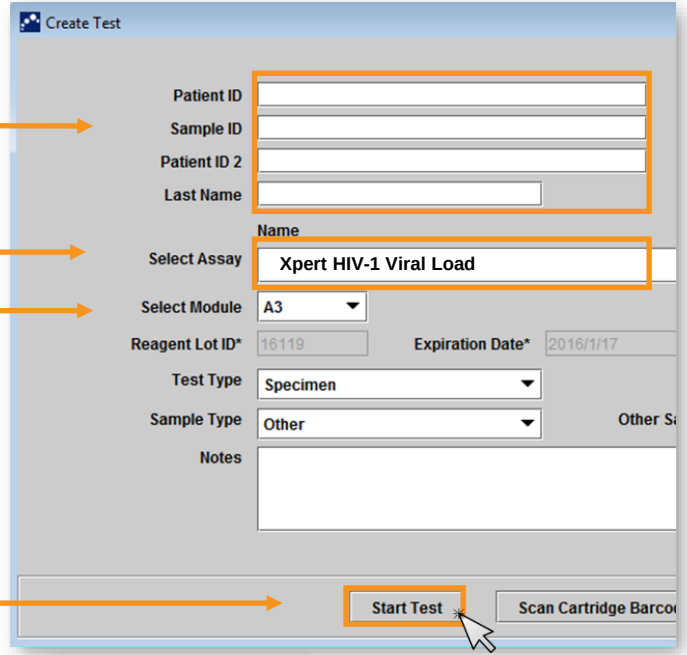
4 Complete the fields as required

5 The Assay Protocol is selected automatically

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

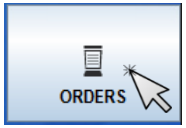
7 Click on Start Test

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



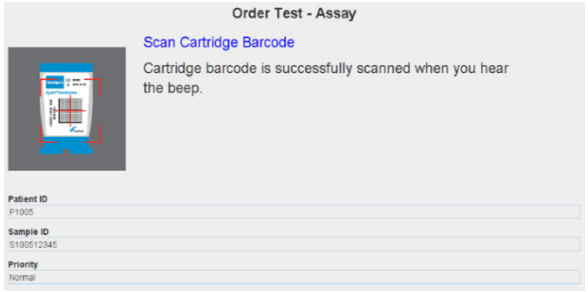
Run a Test on GeneXpert® Infinity

1 Create a test.

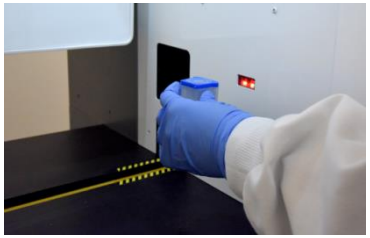


Place the cartridge on the conveyor within **30 minutes** of after adding the sample into the cartridge .

2 Scan barcode for Patient and/or Sample ID.



3 Scan the cartridge.



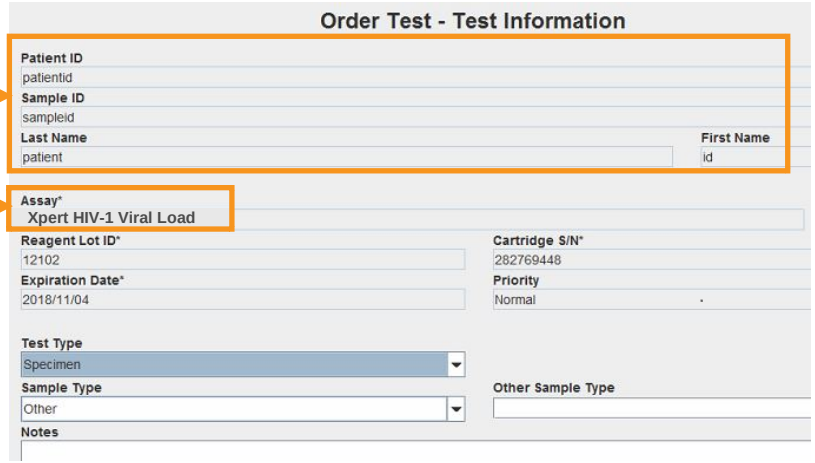
For complete details on how to run a test, refer to the Package Insert and the Xpertise Operator Manual.

Run a Test on GeneXpert® Infinity (continued)

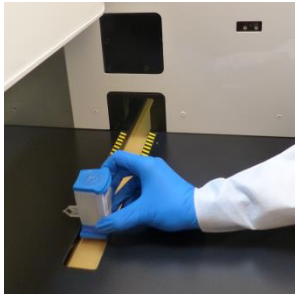
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 Click on SUBMIT



7 Place the cartridge into the conveyor belt



Automated Xpert® HIV-1 Viral Load 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

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Quality Controls

Cepheid Control Strategy

CONTROL

- **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)**
    - **Probe Check Control (PCC)**
    - **Internal Quantitative Standard IQS**

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Internal Quality Controls

- **Sample Volume Adequacy (SVA)**
  - Ensures that the sample was correctly added to the cartridge.
  - The SVA verifies that the correct volume of sample has been added in the sample chamber.
  - The SVA passes if it meets the validated acceptance criteria.
    - If the SVA does not pass :
      an ERROR 2096 will display if there is no sample OR
    - an ERROR 2097 if there is not enough sample. The system will prevent the user from resuming the test.

- **Probe Check Controls (PCC)**
  - Before the PCR step, the fluorescence signal is measured from all probes and compared with default settings to monitor
    - bead rehydration
    - reaction tube filling
    - probe integrity
    - dye stability

Internal Quality Controls continued

- **Internal Quantitative Standard IQS**
  - QS-H and IQS-L are two Armored RNA® controls unrelated to HIV in the form of a dry bead that goes through the whole GX process.
  - The IQS-H and IQSL are standards calibrated against the WHO 3rd International Standard.
  - They are used for quantification by using lot specific parameters for the calculation of HIV-1 RNA concentration in the sample.
  - Additionally, IQS-H and IQS-L detect specimen-associated inhibition of the RT-PCR reaction.
  - The IQS-H and IQS-L pass if they meet the validated acceptance criteria.

Commercially Available External Controls

Thermofisher - https://www.thermofisher.com/order/catalog/product/964001

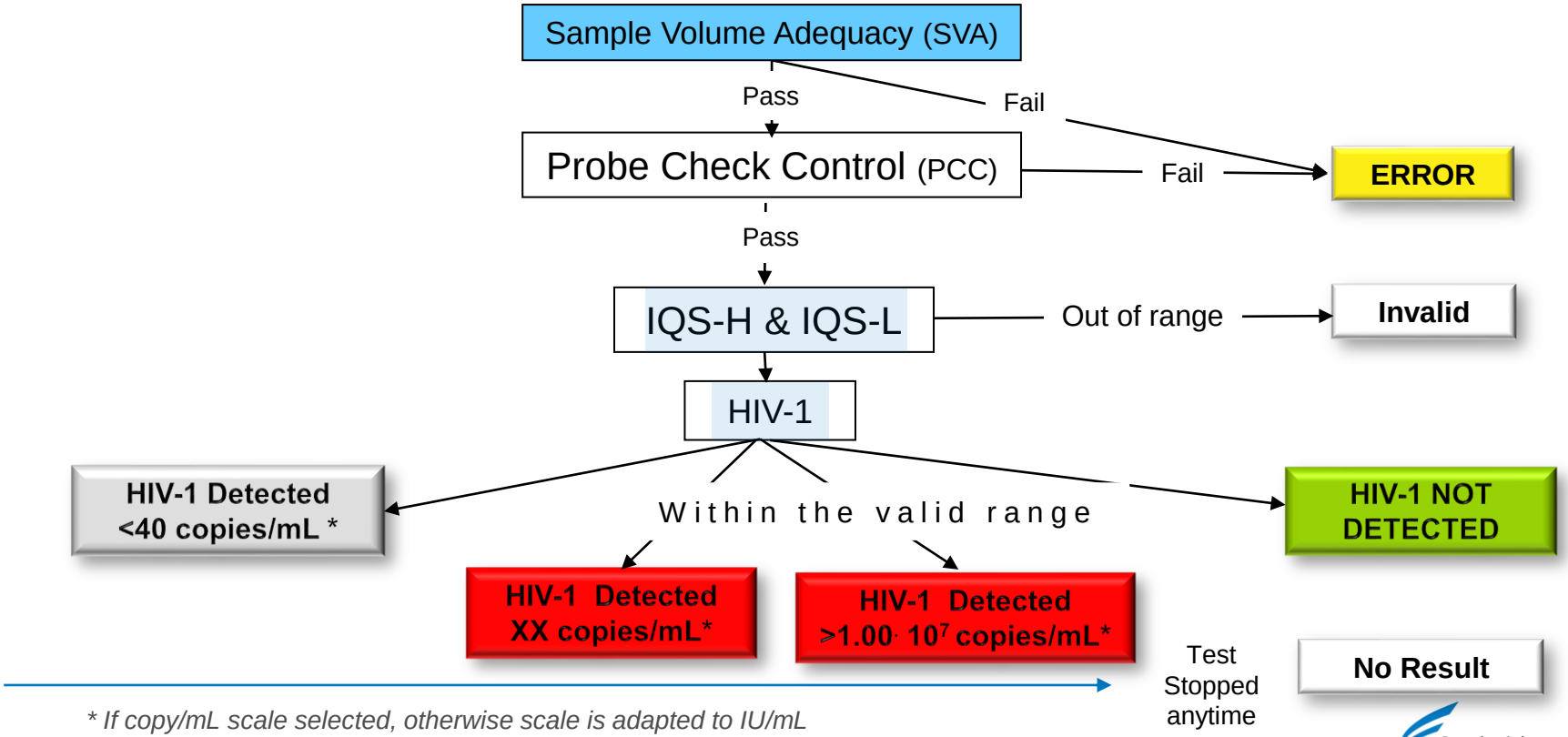
Part Number	Description	Configuration	Storage
964003	HIV-1 High control	1.2 mL x 5 vials	≤ -20°C
964002	HIV-1 Mid control	1.2 mL x 5 vials	≤ -70°C
964001	HIV-1 Low control	1.2 mL x 5 vials	≤ -70°C

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



** If copy/mL scale selected, otherwise scale is adapted to IU/mL*

Copies/mL or IU

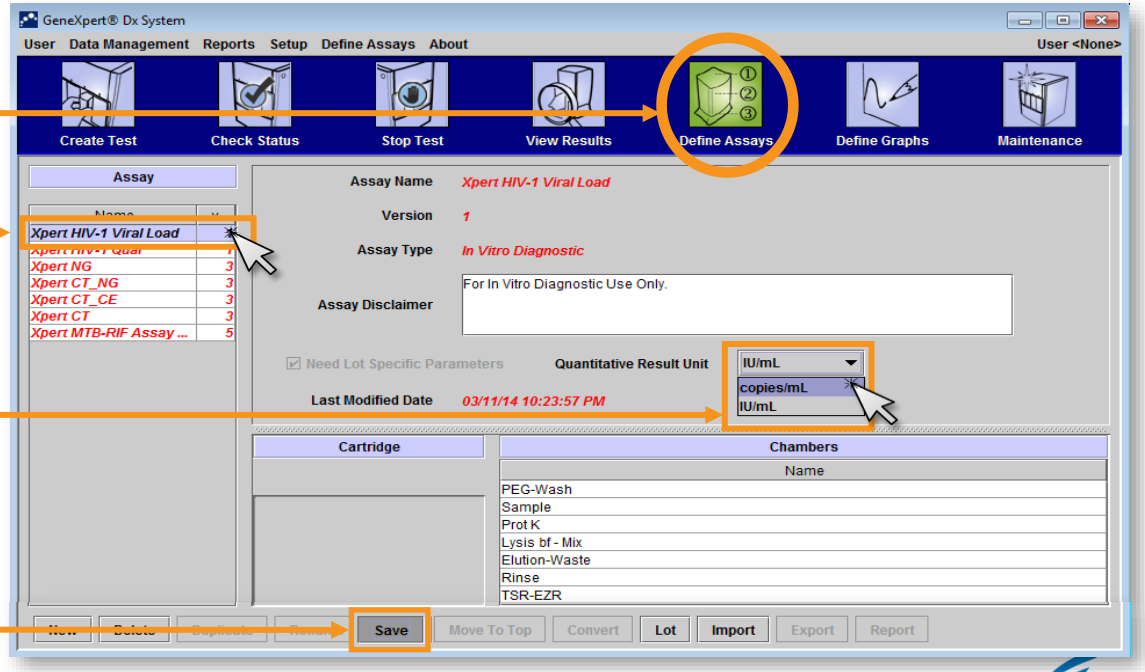
- **You can choose in what units your results are displayed**
  - Copies/mL or International Units/mL *(1 copy/mL = 1.72 IU/mL)*

1 Select Define Assays

2 Highlight Xpert® HIV-1 Viral Load Assay

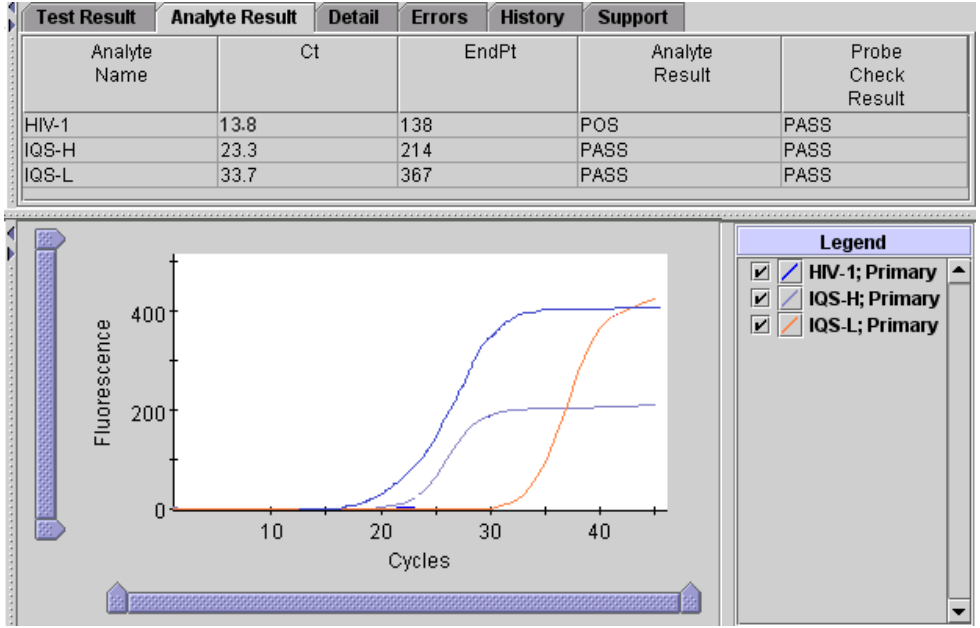
3 Choose units you prefer to see for the quantitative result

4 Save your settings



HIV-1 DETECTED

HIV-1 DETECTED > 1×10^7 copies/mL



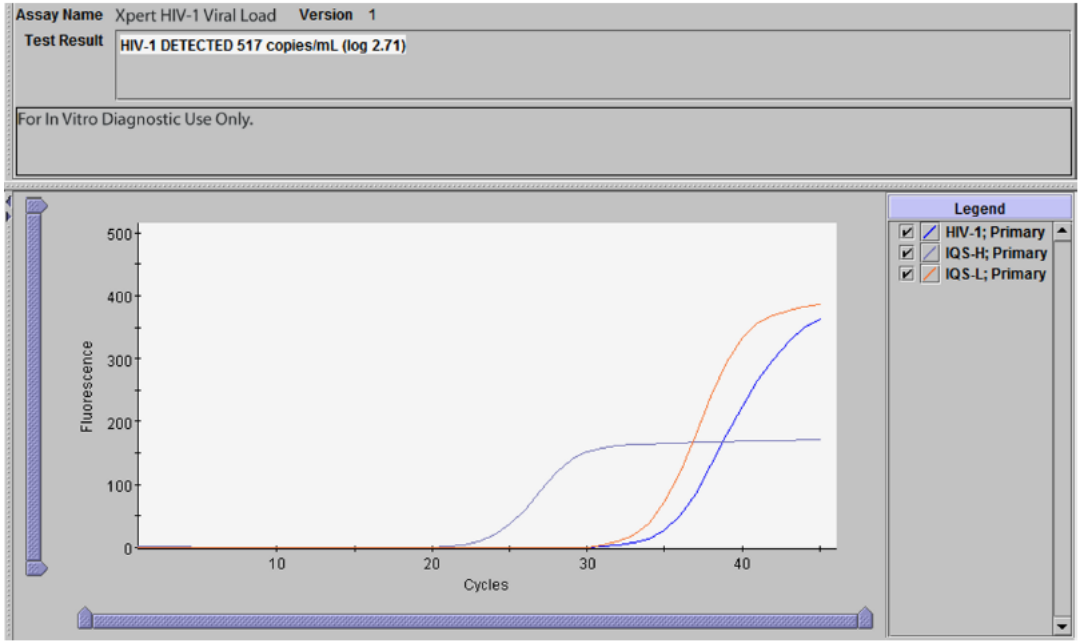
- The target HIV-1 is detected above the analytical measurement range
- IQS-H: PASS
  - IQS-H has a Ct value within the valid range
- IQS-L: PASS
  - IQS-L has a Ct value within the valid range
- Probe Check: PASS

Example calculation:

$1x10^7$ = 10 000 000 (million) copies/mL

HIV-1 DETECTED

HIV-1 DETECTED xx copies/mL



- The target HIV-1 is detected at a quantitative value
- IQS-H: PASS
  - IQS-H has a Ct value within the valid range
- IQS-L: PASS
  - IQS-L has a Ct value within the valid range
- Probe Check: PASS

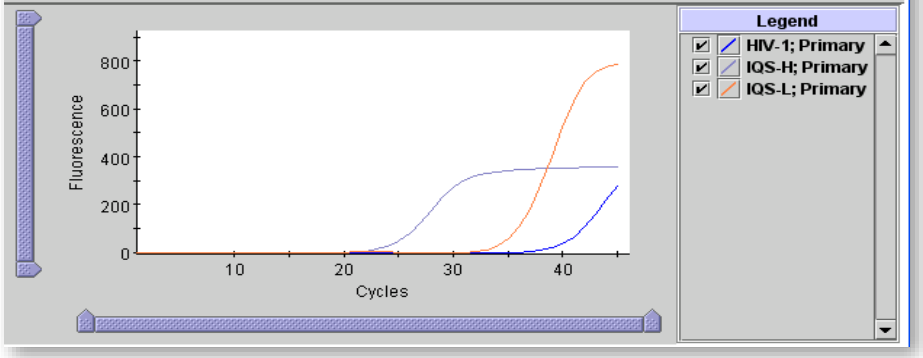
HIV-1 DETECTED

HIV-1 DETECTED < 40 copies/mL

Test Result	HIV-1 DETECTED < 40 copies/mL (log 1.60)

Test Result | Analyte Result | Detail | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
HIV-1	39.4	277	POS	PASS
IQS-H	24.0	359	PASS	PASS
IQS-L	34.6	785	PASS	PASS



- The target HIV-1 is detected below the analytical measurement range
- IQS-H: PASS
  - IQS-H has a Ct value within the valid range
- IQS-L: PASS
  - IQS-L has a Ct value within the valid range
- Probe Check: PASS

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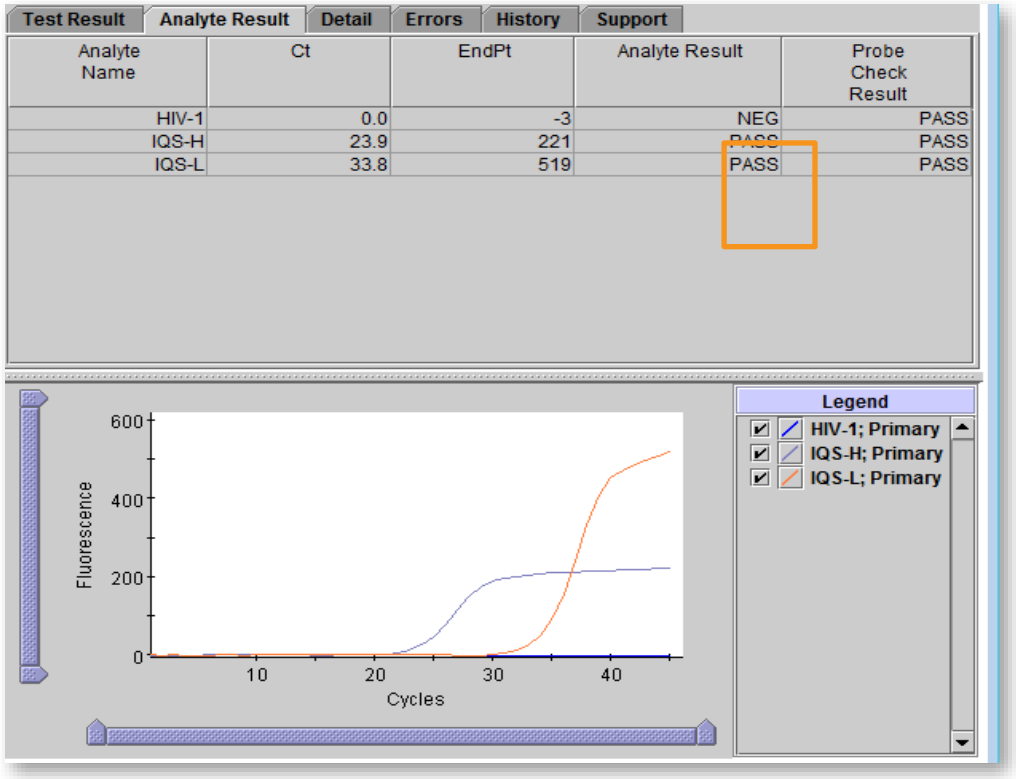
HIV-1 NOT DETECTED

HIV-1 NOT DETECTED



- The target HIV-1 is NOT detected
- IQS-H: PASS
  - IQS-H has a Ct value within the valid range
- IQS-L: PASS
  - IQS-L has a Ct value within the valid range
- Probe Check: PASS

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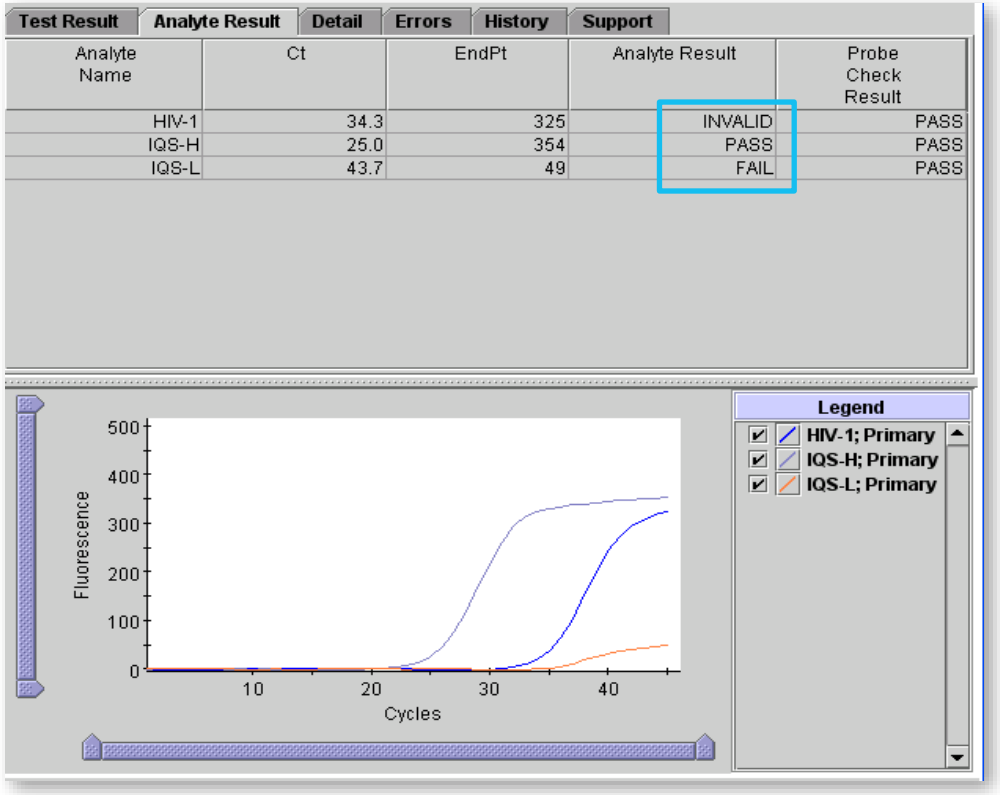
Troubleshooting

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

- IQS-H and or IQS-L: FAIL

  Internal Quantitative Control Cycle thresholds are not within the valid range

- Probe Check: PASS

– Cause
  – Improper sample collection (using heparin tube for e.g)
  – Incorrect sample preparation
  – Improper storage of the cartridges
  – Inefficient sample processing in cartridge
  – Missing primer/probe or enzyme beads
  – Presence of inhibitors in the sample
– Solution
  – Repeat the test with a new cartridge and new sample

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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 detection of HIV-1

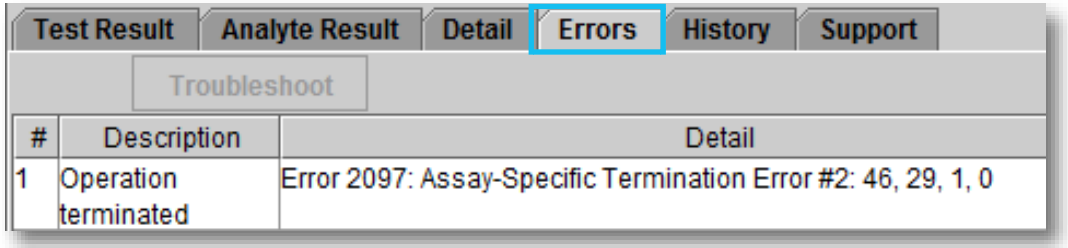
Substance	Tested Concentration
Albumin (BSA)	90 mg/mL
Bilirubin	0.2 mg/mL
Hemoglobin	5 mg/mL
Human DNA	4 µg/mL
Triglycerides	30 mg/mL

  - The drug components below were shown not to interfere with the quantitation or the specificity of the Xpert® HIV-1 Viral Load assay

Pool	Drugs
Control	n/a
1	Zidovudine, Saquinavir, Ritonavir, Clarithromycin
2	Abacavir sulfate, Peginterferon 2b, Ribavirin
3	Tenofovir disoproxil fumarate, Lamivudine, (3TC), Indinavir sulfate, Ganciclovir, Valganciclovir HCl, Acyclovir, Raltegravir
4	Stavudine (d4T), Efavirenz, Lopinavir/Ritonavir, Enfuvirtide (T-20), Ciprofloxacin
5	Nevirapine, Nelfinavir mesylate, Azithromycin, Valacyclovir HCl
6	Fosamprenavir Calcium, Interferon alfa-2b

ERROR

ERROR Result

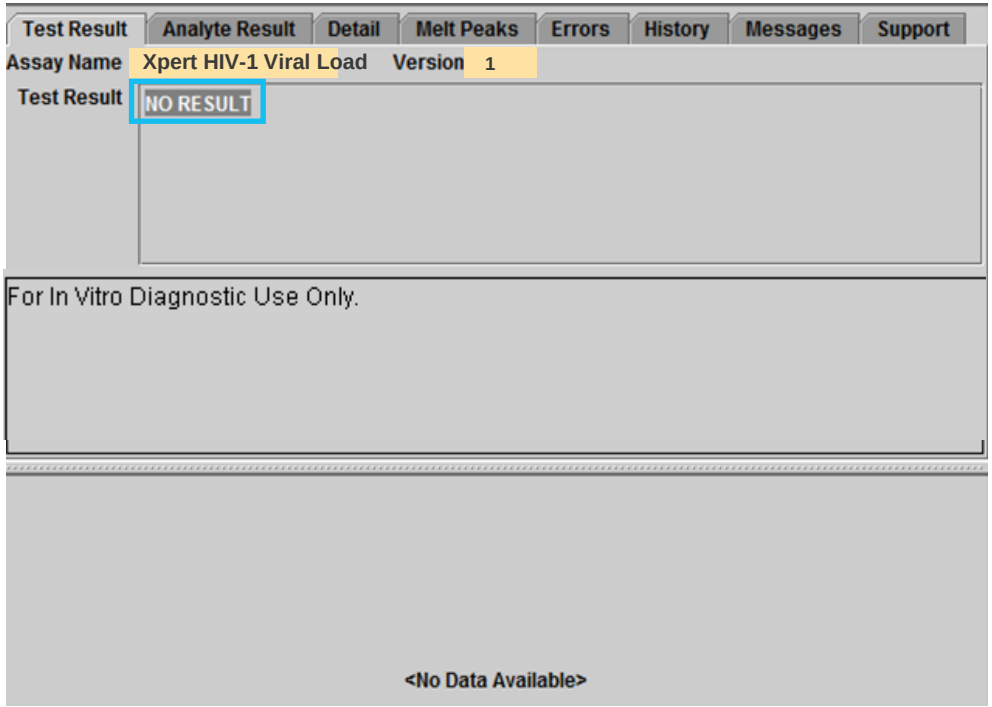


The Sample Volume Adequacy (SVA) passes if it meets the validated acceptance criteria.

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 min. after adding sample

NO RESULT

NO RESULT



- The presence or absence of HIV-1 cannot be determined.
- A NO RESULT indicates that insufficient data were collected.
- IQS-H or IQS-L: NO RESULT
- Probe Check: NA (not applicable)
- **Cause**
  - Test was stopped with stop test button
  - Electrical failure
- **Solution**
  - Secure the power
  - Repeat the test with a new cartridge

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

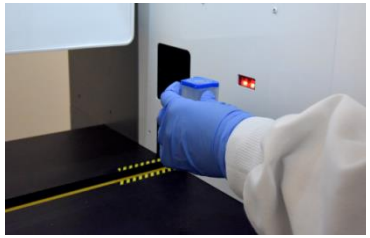
Obtain a new cartridge

Label appropriately as retest on the new cartridge

Process the sample per the package insert

4

Run the test on the GeneXpert® System



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

- Log your complaint online using the following link ***http://www.cepheid.com/en/support:*** *Create a Support Case*

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Thank You

www.Cepheid.com