GeneXpert
Powered By CEPHEID INNOVATION

Xpert® HIV-1 Viral Load XC

REF **GXHIV-VL-XC-CE-10**

Instructions For Use

CE 2797 IVD

Cepheid

In Vitro Diagnostic Medical Device

302-4124, Rev. D
December 2022

Trademark, Patents, and Copyright Statements

See Section 24, Revision History for a description of changes.

Xpert® HIV-1 Viral Load XC

For in Vitro Diagnostic Use Only.

1 Proprietary Name

Xpert® HIV-1 Viral Load XC

2 Common or Usual Name

HIV-1 VL XC

3 Intended Use

Xpert® HIV-1 Viral Load XC (Extended Coverage) is an in vitro reverse transcription polymerase chain reaction (RT-PCR) test for the quantification of human immunodeficiency virus type 1 (HIV-1) RNA in human EDTA plasma using the automated GeneXpert® System.

It is intended for use as an aid in clinical management of patients infected with HIV-1.

Xpert® HIV-1 Viral Load XC 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 from HIV-1 infected individuals.

Xpert® HIV-1 Viral Load XC is intended to be performed by trained professional users or trained healthcare workers in laboratory or near-patient testing environments.

Xpert® HIV-1 Viral Load XC is not intended to be used as a donor screening test for HIV-1 infection.

4 Summary and Explanation

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS). HIV can be transmitted through sexual contact, exposure to infected blood, body fluids, or blood products, prenatal infection of a fetus, or perinatal or postnatal infection of a newborn.

Untreated HIV-1 infection is characterized by high-level viral production and CD4 T-cell destruction, despite an often lengthy clinical latency, to significant net loss of CD4 T cells and AIDS.

HIV diagnostics continue to be important for managing the treatment and care of HIV infected patients. Measurement of blood plasma HIV-1 RNA viral load using nucleic acid-based molecular diagnostic assays has been established as standard of care for assessing HIV-positive patient prognosis and response to antiretroviral therapy. Assessment of viral load levels is a strong predictor of the rate of disease progression and, by itself or in combination with CD4 T-cell counts, has great prognostic value.[1,2]

The HIV-1 VL XC test uses real-time reverse transcription polymerase chain reaction (RT-PCR) technology to achieve high sensitivity for the quantitative detection of HIV-1 RNA in human plasma from HIV-1 infected individuals.

5 Principle of the Procedure

GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex samples using real-time RT-PCR. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require

single-use disposable GeneXpert cartridges that contain the RT-PCR reagents and carry out the sample extraction and RT-PCR 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 System Operator Manual*, *GeneXpert Edge System User's Guide*, or *GeneXpert Infinity System Operator Manual*.

The HIV-1 VL XC test includes reagents for the detection of HIV-1 RNA in samples and two internal controls used for quantitation of HIV-1 RNA. The internal controls are also used to monitor the presence of inhibitor(s) in the RT and PCR reactions. Amplification and detection of HIV-1 RNA is achieved by primers and probes targeted to the highly conserved LTR region and the polymerase gene (dual target) of the HIV-1 genome. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The HIV-1 VL XC test is standardized against the 4th World Health Organization (WHO) International Standard for HIV-1 (NIBSC code 16/194).[3]

6 Materials Provided

The HIV-1 VL XC kit contains sufficient reagents to process 10 samples. The kit contains the following:

HIV-1 VL XC Cartridges with Integrated Reaction Tubes	**10**
Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
Lysis Reagent (Guanidinium Thiocyanate)	2.0 mL per cartridge
Rinse Reagent	0.5 mL per cartridge
Elution Reagent	1.5 mL per cartridge
Binding Reagent	2.4 mL per cartridge
Proteinase K Reagent	0.48 mL per cartridge
Disposable 1 mL Transfer Pipettes	**10 per kit**
CD	**1 per kit**
Assay Definition File (ADF)	
Instructions to import ADF into GeneXpert software	
Instructions for Use (Package Insert)	

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

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

7 Storage and Handling

- Store the HIV-1 VL XC test cartridges at 2–28 °C.
- Prior to use, bring the HIV-1 VL XC test cartridges to 15–30 °C if they have been stored cold.
- Do not open the cartridge lid until you are ready to perform the test.
- Use cartridge within 4 hours after opening the cartridge lid and adding sample.
- Do not use a cartridge that has leaked.
- Do not use cartridges that previously have been frozen.
- Do not use a cartridge past the expiration date.

8 Materials Required but not Provided

- GeneXpert Dx System, GeneXpert Edge System, or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.7b or higher (GeneXpert Dx System), GeneXpert Edge Software Version 1.0 (GeneXpert Edge System) or higher, Xpertise™ 6.4b or higher (GeneXpert Infinity System), barcode scanner, and appropriate GeneXpert System operator manual
- Printer: If a printer is needed, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Bleach or sodium hypochlorite
- Ethanol or denatured ethanol

9 Warnings and Precautions

- For *in vitro* diagnostic use only.
- Treat all biological samples, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological samples should be treated with standard precautions. Guidelines for samples handling are available from the U.S. Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute (CLSI).[4,5]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Appropriate safety measures should be taken in the event of a splash that may occur using bleach and facilities for adequate eye washing or skin rinsing are advised to care for such events.
- Biological samples, 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 disposal. If country or regional regulations do not provide clear direction on proper disposal, biological samples and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.[6]
- Do not substitute HIV-1 VL XC test reagents with other reagents.
- 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 lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label.
- Each single-use HIV-1 VL XC test cartridge is used to process one sample. Do not reuse spent cartridges.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use disposable pipette is used to transfer one sample. Do not reuse spent disposable pipettes.
- If using a precision pipette: Each single use disposable pipette tip is used to transfer one sample. Do not reuse spent pipette tips.
- Wear clean lab coats and gloves. Change gloves between processing each sample.
- In the event of contamination of the work area or equipment with samples, thoroughly clean the contaminated area with a freshly prepared solution of 0.5% sodium hypochlorite (or a 1:10 dilution of household chlorine bleach). Follow by wiping the surface with 70% ethanol. Let the work surfaces dry completely before proceeding.
- For Instrument System cleaning and disinfecting instructions, refer to the appropriate *GeneXpert Dx System Operator Manual*, *GeneXpert Edge System User’s Guide*, or *GeneXpert Infinity System Operator Manual*.

10 Chemical Hazards[7,8]

Signal Word: WARNING

UN GHS Hazard Statements

- Harmful if swallowed.
- Causes mild skin irritation.
- Causes eye irritation.

UN GHS Precautionary Statements
Prevention

- Wash thoroughly after handling.

Response

- Call a POISON CENTER or doctor/physician if you feel unwell.
- If skin irritation occurs: Get medical advice/attention.
- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- If eye irritation persists: Get medical advice/attention.

11 Sample Collection, Transport, and Storage

Whole blood should be collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods, or in sterile collection tubes using K2 EDTA as the anticoagulant. Whole blood should be centrifuged to separate the plasma and red blood cells per the manufacturer's instructions.

- A minimum of 1 mL plasma is required for the HIV-1 VL XC test. If using the transfer pipette included in the kit, fill the transfer pipette with plasma to just below the bulb to transfer the required volume. Alternatively, if using a precision pipette, a minimum of 1 mL plasma is required. See Section 12.2 Preparing the Cartridge, step 6.
- Prior to plasma separation, whole blood collected in BD Vacutainer PPT Plasma Preparation Tubes for Molecular Diagnostic Test Methods, or in sterile collection tubes using K2 EDTA as the anticoagulant may be held at 2–30 °C for up to 24 hours.
- Plasma should be removed from the primary collection tube after centrifugation for storage. Plasma separated from whole blood may be held in secondary tubes at 2–35 °C for up to 24 hours, at 2–8 °C for up to 7 days or frozen (≤ -18 °C and ≤ -70 °C) for up to 6 weeks prior to testing.
- Plasma samples are stable for up to five freeze/thaw cycles. Thaw sample at 15–30 °C.
- Transportation of whole blood or plasma samples must comply with country, federal, state and local regulations for the transportation of etiologic agents.

12 Procedure

12.1 Preparing the Sample

1. Following centrifugation of whole blood samples, plasma may be pipetted directly into the test cartridge. Sufficient volume is critical to obtaining valid test results (see Section 12.2 Preparing the Cartridge).
2. Completely thaw and equilibrate frozen plasma samples to 15–30 °C prior to testing.
3. Remove plasma samples stored at 2–8 °C from the refrigerator and equilibrate to 15–30°C prior to testing.
4. Vortex plasma samples stored at 2–8 °C or frozen and thawed for 15 seconds before use.
5. If the plasma samples are cloudy, clarify by a quick (10 second) centrifugation before use.

12.2 Preparing the Cartridge

Note When using the GeneXpert Dx System or GeneXpert Edge System, start the test within 4 hours of adding the sample to the cartridge. If using a GeneXpert Infinity System, be sure to start the test and put the cartridge on the conveyor within 30 minutes of adding the Sample Reagent-treated sample to the cartridge. Remaining shelf-life is tracked by the Xpertise Software so that tests are run prior to the 4-hour onboard expiration.

Note Pipetting no plasma or less than 1 mL of plasma into the cartridge will trigger an insufficient volume error (ERROR 2096 and ERROR 2097 respectively) preventing the instrument from running the sample.

1. Wear protective disposable gloves.
2. Allow HIV-1 VL XC test cartridges and sample to equilibrate to 15–30 °C prior to pipetting plasma into the cartridge.
   - Do not pipette plasma into a cartridge that is cold (below 15°C).
3. Inspect the test cartridge for damage. If damaged, do not use it.
4. Label the cartridge with sample identification.
5. Open the lid of the test cartridge.
6. Add the sample to the test cartridge.

- If using the *transfer pipette* included in the kit (Figure 1), fill the pipette to just below the bulb to transfer at least 1 mL plasma from the tube (Figure 1). Make sure no large air bubbles are created in the pipette tip while filling the pipette. Empty the contents of the pipette into the sample chamber of the cartridge (Figure 2).
- If using a *precision pipette*, pre-wet the pipette tip once, by filling the pipette tip with plasma and emptying it into the tube. Then, using the pre-wet pipette tip, fill the pipette with at least 1 mL plasma from the tube. Empty the contents of the pipette into the sample chamber of the cartridge (Figure 2).

Note Do not remove the thin plastic film that covers the inner ring of the cartridge.

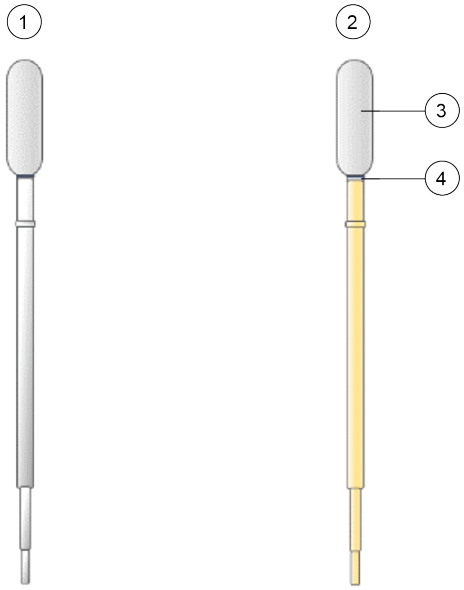


Figure 1. Transfer Pipette

Number	Description
1	Empty pipette
2	Filled pipette
3	Bulb
4	Fill plasma to just below the bulb.

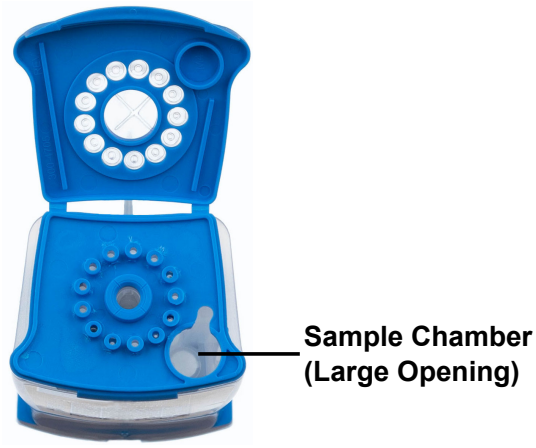


Figure 2. Cartridge (Top View)

7. Close the cartridge lid. Ensure the lid snaps firmly into place.

13 Running the Test

- For the GeneXpert Dx System, see Section 13.1.
- For the GeneXpert Edge System, see Section 13.2.
- For the GeneXpert Infinity System, see Section 13.3.

13.1 GeneXpert Dx System

13.1.1 Starting the Test

Before you start the test, make sure that:

Important

- The system is running the correct GeneXpert Dx software version shown in section - Materials Required but Not Provided.
- The correct 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*.

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert Dx System, then turn on the computer and log on. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on using your username and password.
3. In the **GeneXpert System** window, click **Create Test**.
   The **Create Test** window displays. The **Scan Patient ID barcode** dialog box displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.
   The Patient ID is associated with the test results and displays in the **View Results** window and all the reports. The **Scan Sample ID barcode** dialog box displays.
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 displays in the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box displays.
6. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

7. Click **Start Test**. In the dialog box that displays, type your password, if required.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking.
   When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door, then remove the cartridge.
11. Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

13.1.2 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*.

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.

13.2 GeneXpert Edge System

(May not be available in all countries)

13.2.1 Starting the Test

Important **Before you start the test, make sure that the correct assay definition file (ADF) is imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Edge System User's Guide*.

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Put on a clean pair of gloves.
2. Turn on the GeneXpert Edge instrument. The power switch is on the back of the instrument.
3. Turn on the tablet computer and log on.
   - *Windows 7*: The **Windows 7 account** screen displays. Touch the **Cepheid-Admin** icon to continue.
   - *Windows 10*: The **Windows Lock** screen displays. **Swipe up** to continue.

   The **Windows Password** screen displays.
4. Touch **Password** to display the keyboard, then type your password.
5. Touch the **arrow** button at the right of the password entry area.
   The GeneXpert Edge software loads automatically, and the **Welcome** screen displays shortly thereafter.
6. Touch the **TOUCH HERE TO BEGIN** button.
   The **VIEW PREVIOUS TESTS** button initially displays. The **NEW TEST** button displays on the **Home** screen within 3 minutes when the instrument is ready to run.
7. Touch the **RUN NEW TEST** button on the **Home** screen.
8. Follow the on-screen instructions:
   a) **Scan patient/sample ID** using the barcode scanner, or manually enter the patient/sample ID.
   b) **Confirm the patient/sample ID.**
   c) **Scan the cartridge barcode.**
   The **Select Assay** field automatically fills. Touch **YES** if the displayed information is correct.

Note If the barcode on the cartridge does not scan or scanning the barcode results in an error message, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

   d) **Confirm test** Once the ADF has been selected, confirm the assay.
   e) **Cartridge preparation** The cartridge preparation is also described in the Preparing the Specimen section. Follow the video or instructions on how to prepare the specimen.
   f) **Load cartridge** Open the module door with the blinking green light. Load the cartridge with the barcode facing the operator. Close the door.
   The green light stops blinking, and the test starts. **Test in Progress** displays on the screen.
   g) **Remove cartridge**
   When the test is done (green light goes out), the door automatically unlocks. Follow the displayed instructions on how to remove the cartridge. Dispose of the used cartridge and gloves in an appropriate specimen waste container according to your institution's standard practices.
9. Touch **CONTINUE** to view the result of the test that has just completed. Touch **CONTINUE** again to go back to **Home** screen.
   This completes the procedure for running a test.

13.2.2 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 Edge System User's Guide*.

Note If reporting results using a LIS, confirm that LIS results match system results for the patient ID field; if results conflict, report the system results only.

1. Touch the **VIEW PREVIOUS TESTS** button on the **Home** screen.
2. On the **Select Test** screen, select the test by either touching the test name or using the arrows to select the test.

13.3 GeneXpert Infinity System

13.3.1 Starting the Test

Before you start the test, make sure that:

Important

- The system is running the correct Xpertise software version shown in section - Materials Required but Not Provided.
- The correct assay definition file is imported into the software.

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Infinity System Operator Manual*.

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Power up the instrument. The Xpertise software will launch automatically. If it does not, double-click the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the computer, then log on to the GeneXpert Xpertise software using your user name and password.
3. In the **Xpertise Software Home** workspace, click **Orders** and in the **Orders** workspace, click **Order Test**. The **Order Test - Patient ID** workspace displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and displays in the **View Results** window and all the reports.
5. Enter any additional information required by your institution, and click the **CONTINUE** button. The **Order Test - Sample ID** workspace displays.
6. 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 displays in the **View Results** window and all the reports.
7. Click the **CONTINUE** button. The **Order Test - Assay** workspace displays.
8. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

   After the cartridge is scanned, the **Order Test - Test Information** workspace displays.
9. Verify that the information is correct, and click **Submit**. In the dialog box that displays, type your password, if required.
10. Place the cartridge on the conveyor belt. The cartridge automatically loads, the test runs, and the used cartridge are placed into the waste container.

13.3.2 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 Infinity System Operator Manual*.

1. In the **Xpertise Software Home** workspace, click the **RESULTS** icon. The Results menu displays.
2. In the Results menu, select the **VIEW RESULTS** button. The **View Results** workspace displays showing the test results.
3. Click the **REPORT** button to view and/or generate a PDF report file.

14 Quality Control

Each test includes a Sample Volume Adequacy (SVA) control, Internal Quantitative Standard High and Low (IQS-H and IQS-L), Lot Specific Parameters (LSP), and a Probe Check Control (PCC).

- **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 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 test to be processed.
- **Internal Quantitative Standard High and Low (IQS-H and IQS-L)**: IQS-H and IQS-L are two Armored RNA® controls unrelated to HIV that are included in each cartridge and go through the whole test process. They are used for quantitation 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, thereby acting as sample processing controls. The IQS-H and IQS-L pass if Cycle thresholds (Cts) are within valid range.
- **Lot Specific Parameters (LSP) for Quantitation** – Each kit lot has built-in LSP generated from an HIV-1 calibration panel, traceable to the 4th WHO International Standard for HIV-1 (NIBSC code 16/194), and the IQS-H and IQS-L. The LSP are unique for each kit lot and are used to ensure correct quantitation.
- **Probe Check Control (PCC)**: Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if the fluorescence signals meet the assigned acceptance criteria.

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 (Figure 3 to Figure 11). Possible results are shown in Table 1.

Table 1. Results and Interpretation

Result	Interpretation
HIV-1 DETECTED **XX copies/mL (log X.XX)** See Figure 3 and Figure 9.	HIV-1 RNA is detected at XX copies/mL (log X.XX) ● HIV-1 RNA has quantitative value within the quantitative range of the test - (40-1x10^7 copies/mL). ● IQS-H and IQS-L: PASS. ● Probe Check: PASS; all probe check results pass.
HIV-1 DETECTED **> 1 × 10 ^{7} copies/mL** See Figure 4.	HIV-1 RNA is detected above the analytical measurement range. ● IQS-H and IQS-L: PASS. ● Probe Check: PASS. All probe check results pass.
HIV-1 DETECTED **< 40 copies/mL** See Figure 5.	HIV-1 RNA is detected below the analytical measurement range. ● IQS-H and IQS-L: PASS. ● Probe Check: PASS. All probe check results pass.
HIV-1 NOT DETECTED See Figure 6 and Figure 10.	HIV-1 RNA is not detected. This result does not infer that the patient has been cleared of the virus. ● IQS-H and IQS-L: PASS. ● Probe Check: PASS. All probe check results pass.
INVALID See Figure 7.	Presence or absence of HIV-1 RNA cannot be determined. Repeat test according to the instructions in Section 16.2. ● IQS-H and/or IQS-L: FAIL; Cycle thresholds (Cts) are not within valid range. ● Probe Check: PASS. All probe check results pass.
ERROR See Figure 8.	Presence or absence of HIV-1 RNA cannot be determined. Repeat test according to the instructions in Section 16.2. ● Probe Check: FAIL; all or one of the probe check results fail.

Result	Interpretation
NO RESULT See Figure 11.	Presence or absence of HIV-1 RNA cannot be determined. Repeat test according to the instructions in Section 16.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Note Results can be converted from copies/mL to IU/mL within the software. Please see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual* for instructions on how to change this setting.

The conversion factor for the HIV-1 VL XC test is 1 copy = 2.06 International Unit (IU).

Note Assay screenshots are for example only. The version number may vary from the screenshots shown in this package insert.

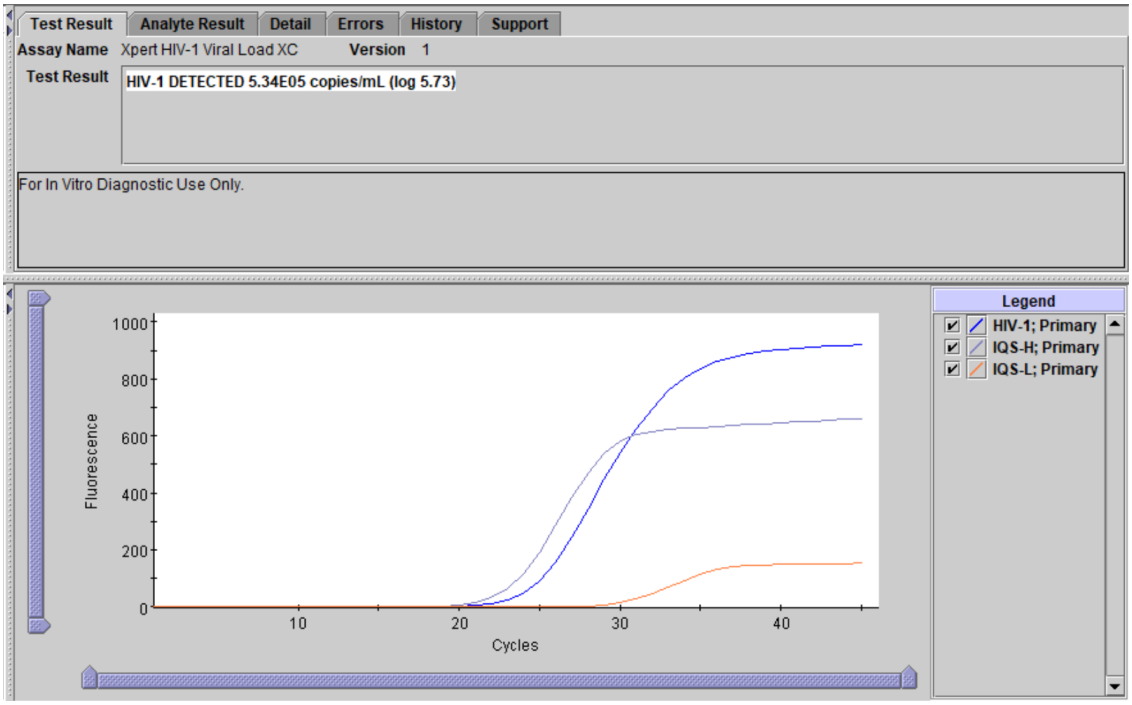


Figure 3. Result: HIV-1 Detected and Quantified (GeneXpert Dx System and GeneXpert Infinity System)

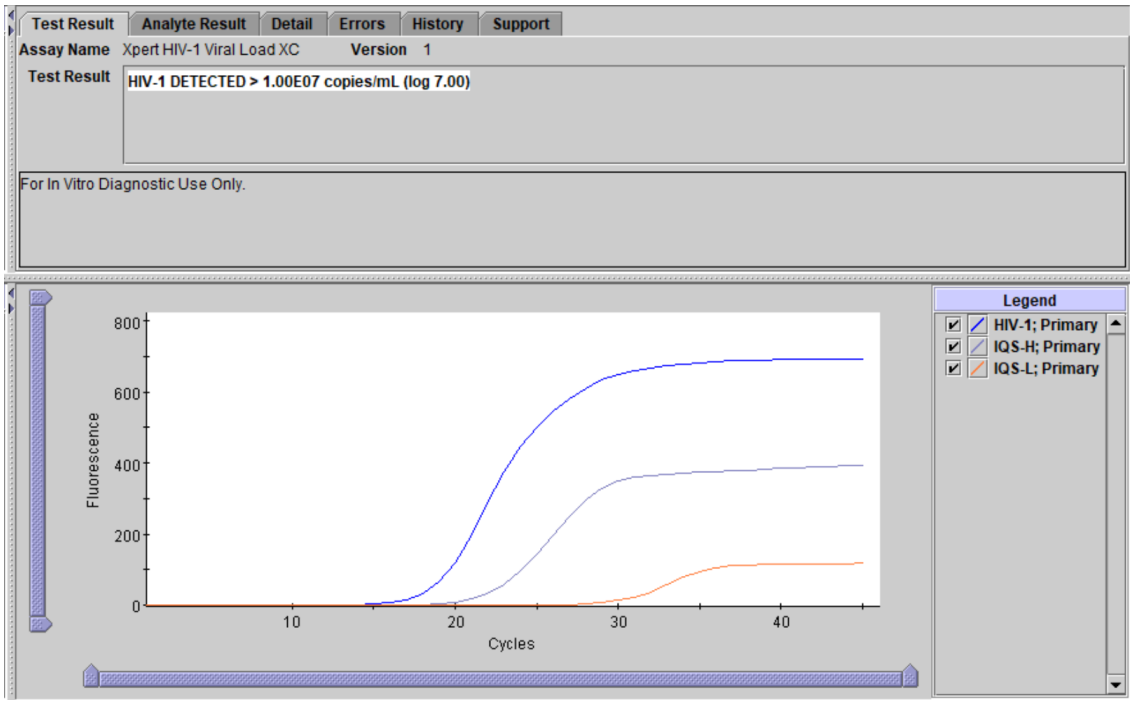


Figure 4. Result: HIV-1 Detected but with titer above the quantitative range of the test (GeneXpert Dx System and GeneXpert Infinity System)

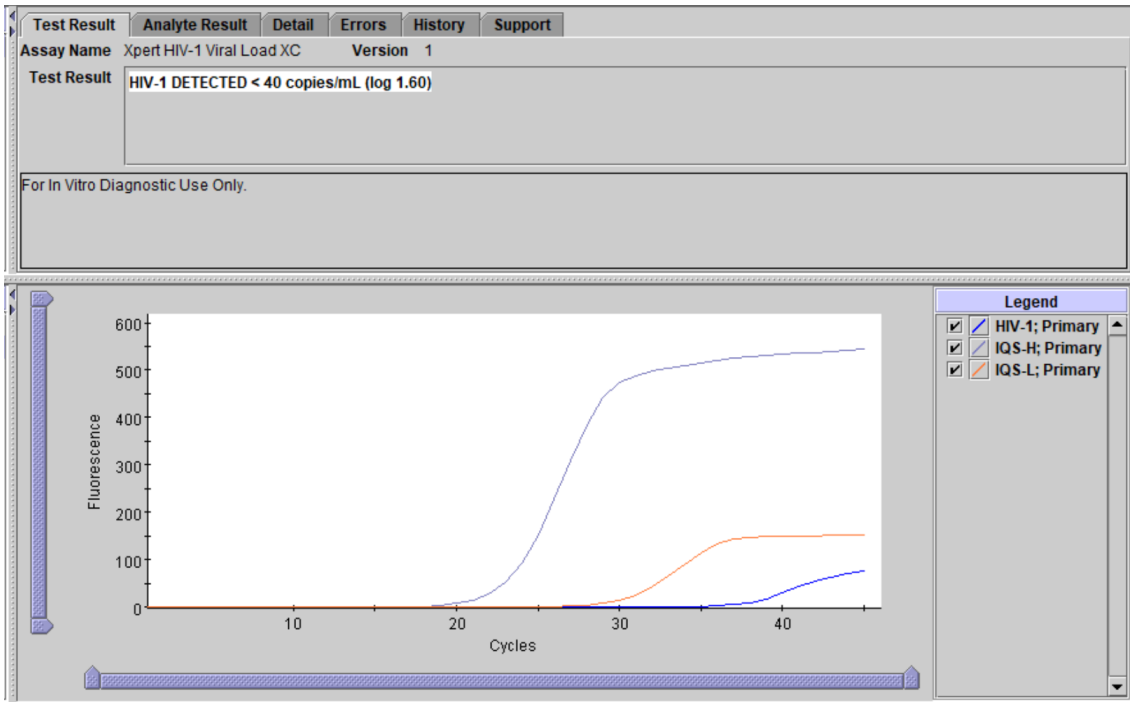


Figure 5. Result: HIV-1 Detected but with titer below the quantitative range of the test (GeneXpert Dx System and GeneXpert Infinity System)

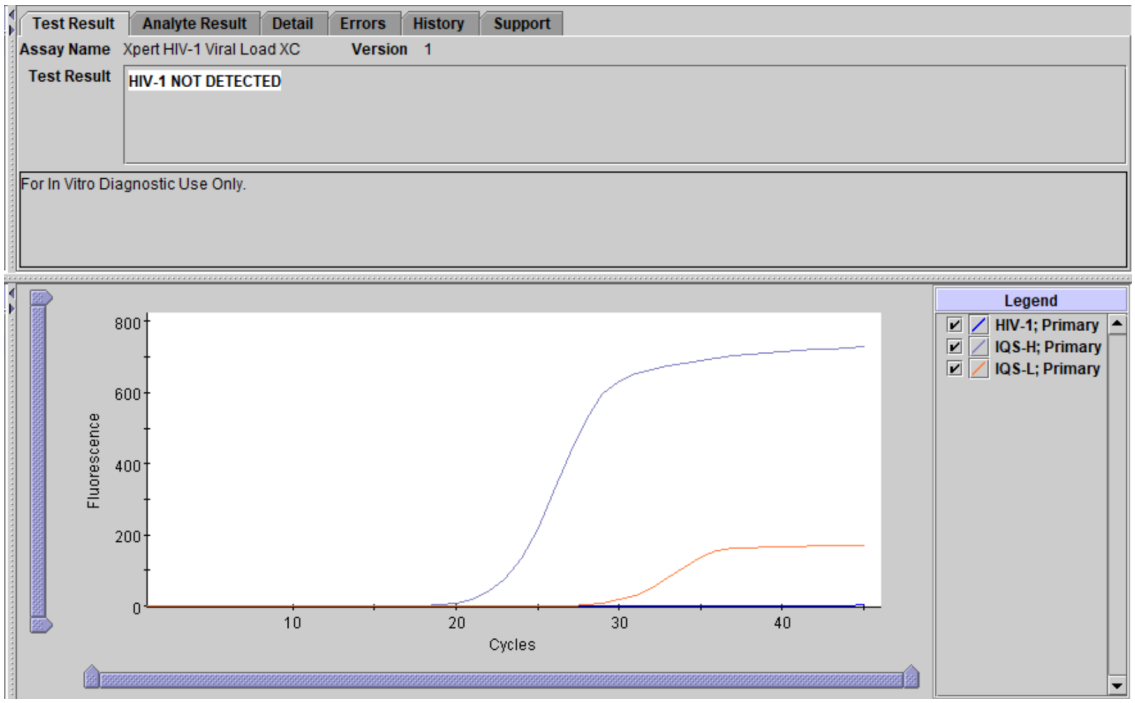


Figure 6. Result: HIV-1 Not detected (GeneXpert Dx System and GeneXpert Infinity System)

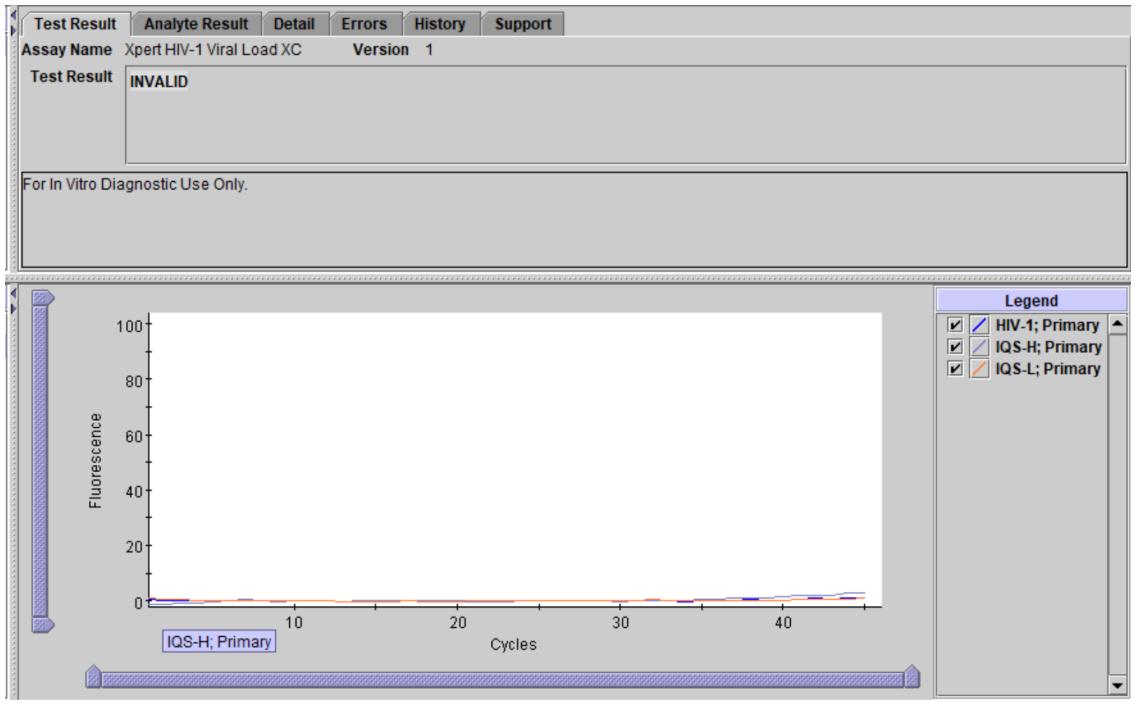


Figure 7. Invalid Result (GeneXpert Dx System and GeneXpert Infinity System)

Figure 8. Result: Error (GeneXpert Dx System and GeneXpert Infinity System)

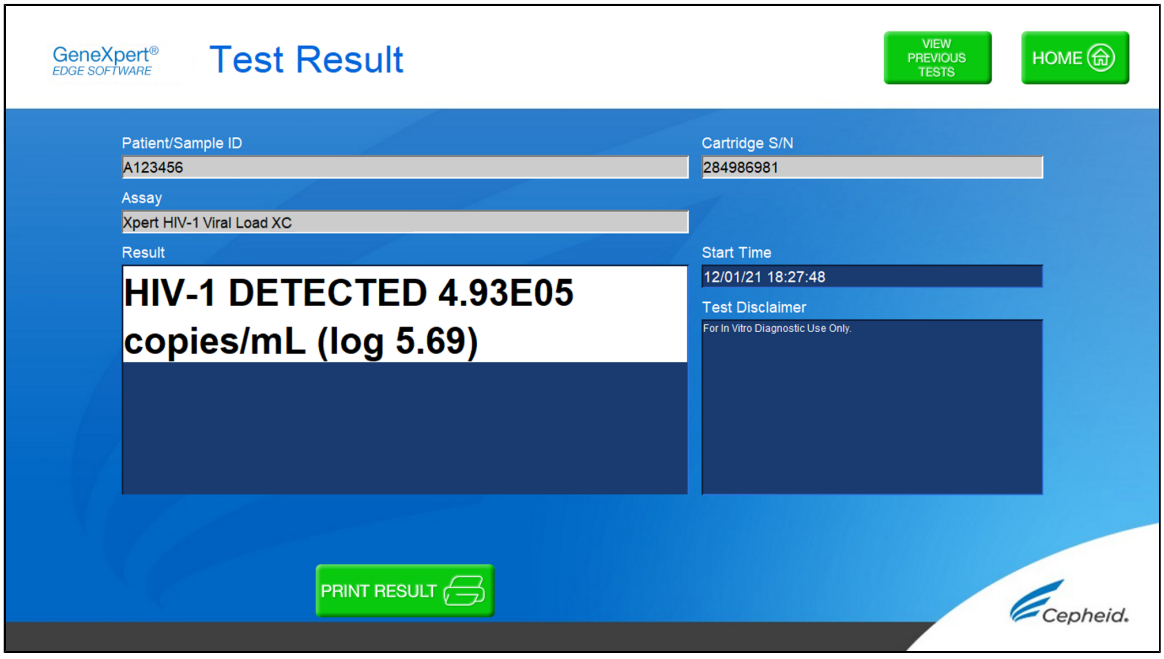


Figure 9. Result: HIV-1 Detected (GeneXpert Edge System)

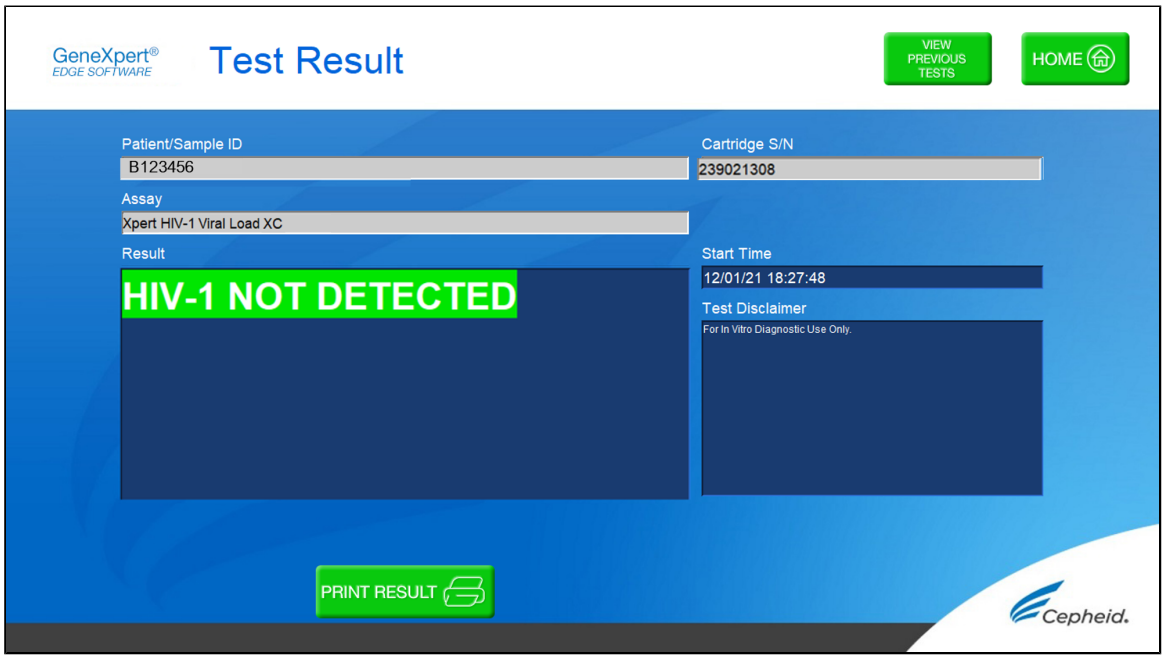


Figure 10. Result: HIV-1 Not Detected (GeneXpert Edge System)

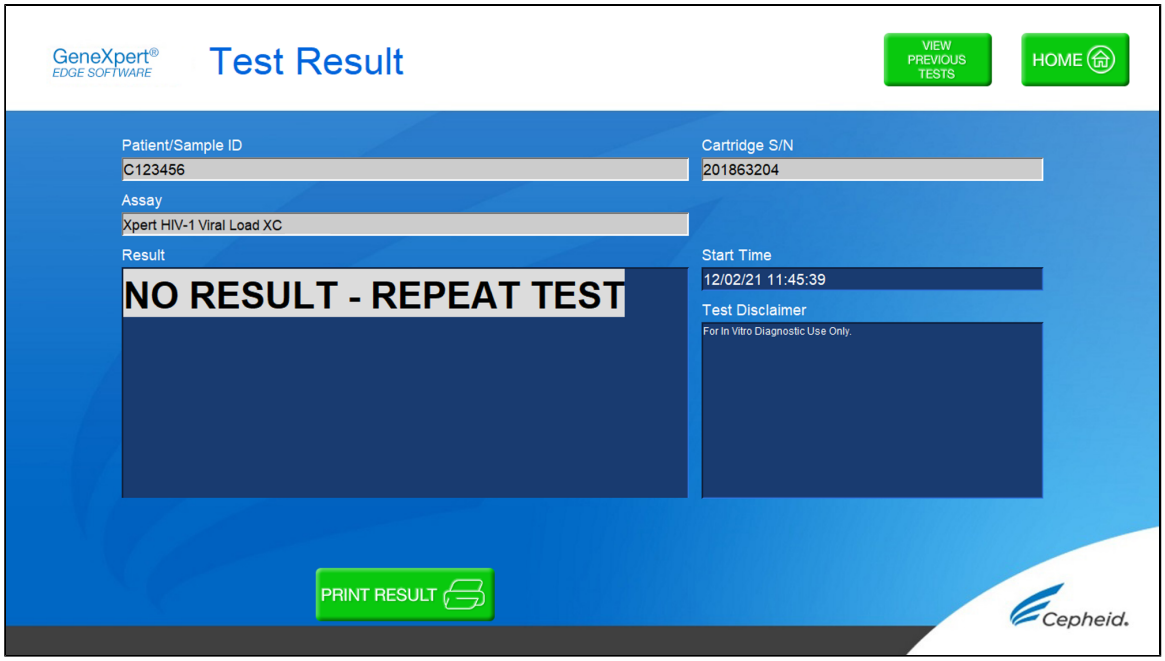


Figure 11. No Result-Repeat Test (GeneXpert Edge System)

16 Retests

16.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test according to instructions in Section 16.2.

- An **INVALID** result indicates one or more of the following:

- The IQS-H and/or IQS-L Cts are not within valid range.
- The sample was not properly processed, or PCR was inhibited.

- An **ERROR** indicates that the test was aborted. Possible causes include: insufficient volume of sample was added, the reaction tube was filled improperly, a reagent probe integrity problem was detected, or 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

If the result of a test is **INVALID**, **ERROR**, or **NO RESULT**, use a new cartridge to retest the affected sample (do not re-use the cartridge).

1. Remove a new cartridge from the kit.
2. Go to Section 12, Procedure, including Section 12.2, Preparing the Cartridge, and Section 12.3, Starting the Test.

17 Limitations

- Good laboratory practice and changing gloves between handling samples are recommended to avoid contamination of samples or reagents.
- Rare mutations, deletions or insertions within the target regions of the HIV-1 VL XC test may affect primer and/or probe binding resulting in under-quantitation or failure to detect the virus.
- 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.
- The HIV-1 VL XC test has been validated only for use with K2 EDTA and PPT-EDTA plasma. Testing of other sample types may lead to inaccurate results.
- A negative test result does not preclude HIV-1 infection. Results from the HIV-1 VL XC test should be interpreted in conjunction with clinical presentation and other laboratory markers.
- Prior to switching from one technology to the next, Cepheid recommends that users perform method correlation studies in their laboratory to qualify technology differences.
- Reliable results are dependent on adequate sample collection, transport, storage and processing.
- Quantitation of HIV-1 RNA is dependent on the number of virus particles present in a sample and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- A sample that yields an **INVALID** result twice may contain an inhibitor; retesting is not recommended.

18 Performance Characteristics

18.1 Analytical Sensitivity (Limit of Detection (LoD) and Inclusivity)

The limit of detection (LoD) of the HIV-1 VL XC test was determined for group M subtype B by testing serial dilutions prepared from the WHO 4th International Standard for HIV-1 (NIBSC code: 16/194) in HIV-1 negative K2 EDTA plasma. In total six different concentration levels of the WHO International Standard and one negative were tested with three kit lots. Each concentration level was tested across three days with 24 replicates per kit lot for a total of 72 replicates per concentration level.

The results are shown in Table 2. The study demonstrated that the HIV-1 VL XC test detected HIV-1 RNA for the WHO International Standard at a concentration of 13.6 copies/mL in K2 EDTA plasma with a positivity rate of 95% as determined by PROBIT regression.

Table 2. Limit of Detection for the HIV-1 VL XC test using the 4th WHO International Standard for HIV-1

Group/Subtype	Nominal HIV-1 Concentration (copies/mL)	Number of Valid Replicates	Number of Positive Replicates	Positivity Rate (%)	LoD with 95% Probability Estimated by Probit (95% Confidence Interval)
Group M/ Subtype B	0	72	0	0	13.6 copies/ mL (11.7-15.6)
	1	72	13	18	
	2.5	72	31	43	
	5	72	45	63	
	10	72	60	83	
	20	72	70	97	
	40	72	72	100	

The limit of detection for the HIV-1 group M subtypes A, C, D, F, G, H, J, K, CRF-A/B, CRF-A/E, CRF-A/G, CRF-B/C, CRF-06, group N, group O and group P was determined by testing serial dilutions of cell culture stocks or clinical specimens representing each HIV-1 group and subtype in HIV-1 negative K2 EDTA plasma. In total six concentration levels of each HIV-1 group and subtype was tested with one kit lot across three days for a total of 24 replicates per concentration level.

The assignment of the nominal concentration of the cell culture stocks and clinical specimens was determined using CE-marked HIV-1 viral load tests.

The HIV-1 RNA concentration that can be detected with a positivity rate of 95% was determined by PROBIT regression. The results for each HIV-1 group M subtypes A, C, D, F, G, H, J, K, CRF-A/B, CRF-A/E, CRF-A/G, CRF-B/C, CRF-06, group N, group O and group P are shown in Table 3.

Table 3. Limit of Detection for HIV-1 group M subtypes A, C, D, F, G, H, J, K, CRF-A/B, CRF-A/E, CRF-A/G, CRF-B/C, CRF-06, group N, group O and group P in K2 EDTA Plasma

Group	Subtype	LoD by PROBIT (copies/mL)	95% Confidence Interval (copies/mL)
Group M	A	15.9	12.1-19.7
	C	13.2	10.2-16.3
	D	17.7	13.5-21.8
	F	18.1	14.5-21.6
	G	18.0	13.7-22.3
	H	7.9	6.2-9.5
	J	14.2	10.6-17.7
	K	16.9	12.7-21.0
	CRF A/B	13.1	9.9-16.3
	CRF A/E	14.2	10.7-17.6
	CRF A/G	17.4	13.2-21.6
	CRF B/C	17.0	13.3-20.8
	CRF 06	10.8	8.4-13.2
Group N	N/A	16.5	12.2-20.8

Group	Subtype	LoD by PROBIT (copies/mL)	95% Confidence Interval (copies/mL)
Group O	N/A	9.0	6.8-11.1
Group P	N/A	4.9	3.9-5.9

18.2 Limit of Quantitation (LoQ)

The lower limit of quantitation (LLoQ) is defined as the lowest concentration of HIV-1 RNA that is quantified with acceptable precision and trueness and is determined using the total analytical error (TAE) and an approach based upon the difference between two measurements. The TAE for HIV-1 VL XC was calculated using estimates determined through analysis of data from the LoD study (WHO International Standard) and data from testing performed on three HIV-1 subtype B clinical specimens in K2 EDTA plasma (value assigned with a CE-marked HIV-1 Viral Load test) at a concentration of 40 HIV-1 RNA copies/mL using two kit lots with 16 replicates per kit lot.

TAE was estimated with the Westgard model according to CLSI guideline with the criterion, [(Absolute Bias) + 2 SDs) ≤ 1 $\log_{10}$ copies/mL].[9] The difference between two measurements approach was evaluated with the criterion, [(2 x SQRT(2) x SD) ≤ 1 $\log_{10}$ copies/mL].

The LLoQ analyses for each specimen are presented in Table 4. The result demonstrates that the HIV-1 VL XC test can determine 40 copies/mL HIV-1 RNA with an acceptable trueness and precision.

Table 4. Determination of the LLoQ for the HIV-1 VL XC Test

HIV-1 Subtype B Sample	Kit Lot	N	Nominal HIV-1 Conc. ($\log_{10}$ copies/mL)	Observed HIV-1 Conc. ($\log_{10}$ copies/mL)	Bias	Total SD	Total Analytical Error[a]	Two Measurement Approach[b]
WHO	1	24	1.60	1.51	-0.09	0.14	0.37	0.39
	2	24	1.60	1.48	-0.12	0.17	0.47	0.49
	3	24	1.60	1.56	-0.04	0.31	0.65	0.87
Clinical Specimen 1	1	16	1.60	1.65	0.05	0.10	0.25	0.29
	2	16	1.60	1.63	0.03	0.11	0.25	0.32
Clinical Specimen 2	1	16	1.60	1.80	0.20	0.12	0.44	0.35
	2	16	1.60	1.73	0.13	0.12	0.37	0.34
Clinical Specimen 3	1	16	1.60	1.45	-0.15	0.29	0.72	0.81
	2	16	1.60	1.62	0.02	0.16	0.33	0.45

[a] TAE calculated according to the Westgard model where [TAE = $|\text{Bias}| + (2\times\text{SD}) \le 1 \log_{10}$ copies/mL] ensuring there is a 95% probability that the measurement will be less than 1 $\log_{10}$ copies/mL from the true value.

[b] Two measurements approach [2 × (SQRT(2) × SD) ≤ 1 $\log_{10}$ copies/mL] indicates that a difference of less than 1 $\log_{10}$ copies/mL can be explained by a random measurement error.

18.3 Precision and Reproducibility

The precision and reproducibility of the HIV-1 VL XC test was established in a three-site, blinded study using a seven-member panel of HIV-1 reference material spiked into HIV-1 negative EDTA plasma with RNA concentrations that span the HIV-1 VL XC test quantitation range. Two operators at each of the three study sites tested one panel of seven samples twice per day over six testing days. Two sites used GeneXpert Dx instruments and one site used an Infinity-80 instrument. Three kit lots of the HIV-1 VL XC test were used in the study. The precision/reproducibility study was evaluated in accordance with CLSI guideline.[10]

The reproducibility of the HIV-1 VL XC test was evaluated by using nested ANOVA with terms for Site/Instrument, Lot, Operator, Day, Run, and Within-Run. The standard deviation and the percentage of variability due to each component of the log_{10} HIV-1 transformed concentrations were calculated (see Table 5).

Table 5. HIV-1 VL XC Test Contribution to Total Variance and Total Precision

Expected HIV-1 RNA Concentration (copies/mL)	N	Mean[a]	Variance Source													
			Site		Lot		Operator		Day		Run		Within-Run		Total	
			SD[b]	(%)	SD	(%)	SD	(%)	SD	(%)	SD	(%)	SD	(%)	SD	CV (%)[c]
40 cp/mL	143[d]	1.59	0.01	0.55	0.03	2.15	0.04	5.97	0.05	7.80	0.00	0.00	0.16	83.53	0.17	10.69
200 cp/mL	144	2.28	0.02	5.52	0.03	9.27	0.01	2.08	0.00	0.00	0.00	0.00	0.09	83.14	0.10	4.39
$1x10^3$ cp/mL	144	2.99	0.00	0.00	0.02	9.75	0.00	0.00	0.02	13.86	0.00	0.00	0.06	76.38	0.06	2.01
$1x10^4$ cp/mL	144	3.98	0.01	4.72	0.02	15.66	0.00	0.00	0.00	1.00	0.01	6.19	0.04	72.43	0.05	1.26
$1x10^6$ cp/mL	143[e]	6.01	0.01	3.40	0.03	15.35	0.00	0.00	0.00	0.00	0.00	0.00	0.06	81.25	0.07	1.16
$1x10^7$ cp/mL	144	6.96	0.00	0.00	0.04	17.70	0.00	0.00	0.03	10.97	0.00	0.00	0.09	71.32	0.10	1.44

[a] Mean HIV-1 RNA cp/mL log_{10}
[b] SD in log_{10}
[c] CV = (total SD/mean)*100
[d] 1 sample with “HIV-1 Not Detected” result was excluded
[e] 1 sample with “Error” result was excluded

18.4 Linear Range

The linear range of the HIV-1 VL XC test was determined by analysis of a nine-member panel ranging from 15 copies/mL to 1.2 x 10^7 copies/mL prepared by parallel dilutions of HIV-1 reference material (HIV-1 subtype B) in HIV-1 negative K2 EDTA plasma. The reference material used was calibrated to the WHO 4th International standard for HIV-1 (NIBSC code: 16/194). The panel was tested using two kit lots of the HIV-1 VL XC test, resulting in total 24 or 48 replicates per panel member.

The linearity analysis was performed according to CLSI guideline.[11] The results are presented in Figure 12. The HIV-1 VL XC test is linear from 20 copies/mL to $1x10^7$ copies/mL with an R^2>99.

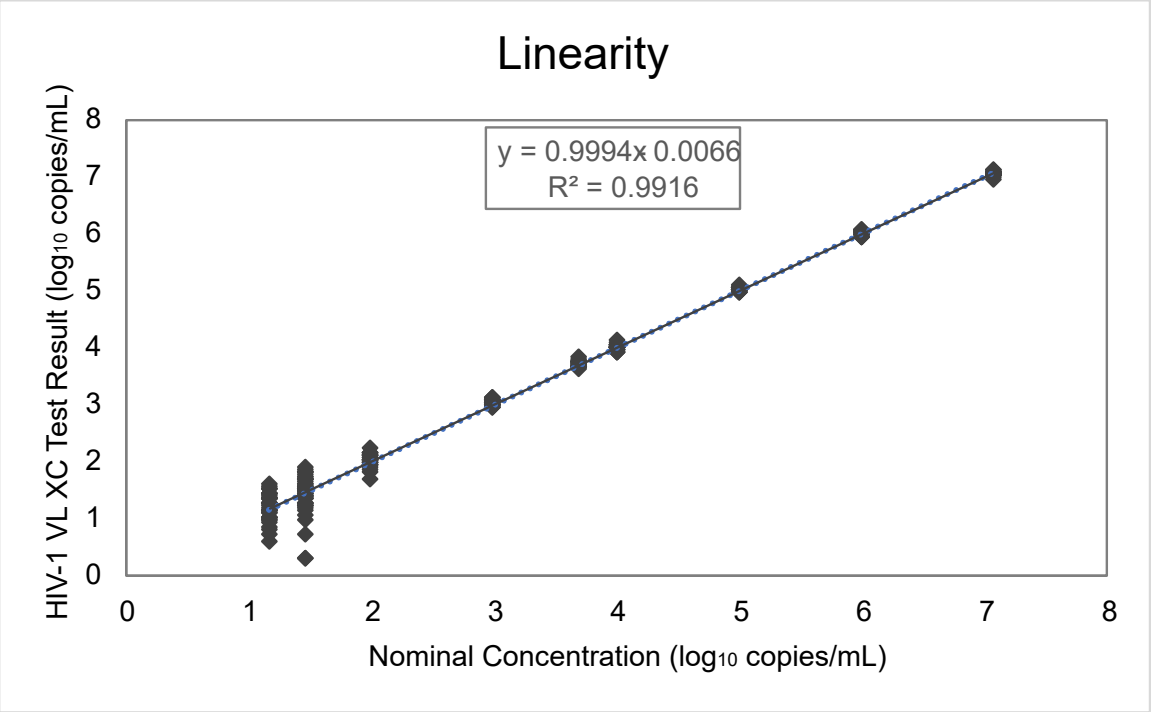


Figure 12. Linearity for the HIV-1 VL XC Test

18.5 Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) for the HIV-1 VL XC test was demonstrated by testing HIV-1 Group M, subtypes A, B, C, D, F, G, H, J, K, CRF-A/B, CRF-A/E, CRF-A/G, CRF-B/C, CRF-06, group N, group O and group P at multiple concentration levels spanning the test's quantitative range of 40-1x10^7 copies/mL depending on subtype/group. Each concentration level was tested in replicates of minimum eight using two kit lots of the HIV-1 VL XC test. The mean $\log_{10}$ concentration obtained for each subtype/group and concentration level was quantified within ± 0.5 $\log_{10}$ of the assigned input concentration and each linear regression had an R2 >0.98 (see Table 6, Table 7, and Table 8).

Table 6. Analytical Reactivity (Inclusivity) for the HIV-1 VL XC Test, HIV-1 Group M subtypes

HIV-1 Group M subtype	Nominal Concentration ($\log_{10}$ copies/mL)	HIV-1 VL XC Result ($\log_{10}$ copies/mL)	Delta ($\log_{10}$ copies/mL)	R^2
A	6.0	5.91	0.09	0.996
	4.0	3.99	0.01	
	2.0	2.02	-0.02	
	1.3	1.37	-0.07	
B	7.0	7.02	-0.02	0.998
	5.0	5.12	-0.12	
	3.0	3.14	-0.14	
	1.3	1.34	-0.04	
C	6.0	5.89	0.11	0.994
	4.0	3.99	0.01	
	2.0	2.03	-0.03	

HIV-1 Group M subtype	Nominal Concentration (log_{10} copies/mL)	HIV-1 VL XC Result (log_{10} copies/mL)	Delta (log_{10} copies/mL)	R^2
	1.3	1.33	-0.03	
D	6.0	5.83	0.17	0.995
	4.0	3.93	0.07	
	2.0	2.00	0.00	
	1.3	1.39	-0.09	
F	6.0	5.74	0.26	0.988
	4.0	3.83	0.17	
	2.0	1.79	0.21	
	1.3	1.12	0.18	
G	6.0	5.89	0.11	0.994
	4.0	3.92	0.08	
	2.0	1.95	0.05	
	1.3	1.16	0.14	
H	5.0	4.92	0.08	0.988
	4.0	3.94	0.06	
	2.0	1.99	0.01	
	1.3	1.52	0.08	
J	2.3	2.36	-0.05	NA[a]
	2.0	2.05	-0.05	
	1.3	1.42	-0.12	
K	4.0	3.86	0.14	0.980
	3.0	2.84	0.16	
	2.0	1.90	0.10	
	1.3	1.11	0.19	

[a] Linear regression analysis was not performed for HIV-1 Group M subtype J and CRF-A/B due to unavailability of specimens spanning a large concentration range.

Table 7. Analytical Reactivity (Inclusivity) for the HIV-1 VL XC Test, HIV-1 CRF's

HIV-1 CRF	Nominal Concentration (log_{10} copies/mL)	HIV-1 VL XC Result (log_{10} copies/mL)	Delta (log_{10} copies/mL)	R^2
CRF-A/B	2.3	2.39	-0.09	NA[a]
	2.0	1.97	0.03	
	1.3	1.32	-0.02	
CRF-A/E	6.0	5.95	0.05	0.992
	4.0	3.97	0.03	
	2.0	1.96	0.04	
	1.3	1.11	0.19	
CRF-A/G	6.0	5.87	0.13	0.991
	4.0	3.90	0.10	
	2.0	1.86	0.14	
	1.3	1.13	0.17	
CRF-B/C	6.0	5.70	0.30	0.995
	4.0	3.74	0.26	
	2.0	1.81	0.19	
	1.3	1.11	0.19	
CRF-06	7.0	6.94	0.06	0.997
	5.0	5.04	-0.04	
	3.0	3.05	-0.05	
	1.3	1.24	0.06	

[a] Linear regression analysis was not performed for HIV-1 Group M subtype J and CRF-A/B due to unavailability of specimens spanning a large concentration range.

Table 8. Analytical Reactivity (Inclusivity) for the HIV-1 VL XC Test, HIV-1 Group N, Group O and Group P

HIV-1 Group	Nominal Concentration (log_{10} copies/mL)	HIV-1 VL XC Result (log_{10} copies/mL)	Delta (log_{10} copies/mL)	R^2
N	7.0	6.78	0.22	0.994
	5.0	4.84	0.16	
	3.0	2.88	0.12	
	1.3	1.26	0.04	
O	6.0	5.96	0.04	0.995
	4.0	4.07	-0.07	
	2.0	2.12	-0.12	
	1.3	1.54	-0.24	
P	5.0	5.17	-0.17	0.996

HIV-1 Group	Nominal Concentration (log_{10} copies/mL)	HIV-1 VL XC Result (log_{10} copies/mL)	Delta (log_{10} copies/mL)	R^2
	4.0	4.21	-0.21	
	2.0	2.21	-0.21	
	1.3	1.51	-0.21	

In addition, analytical reactivity (inclusivity) for the HIV-1 VL XC test was demonstrated by testing HIV-1 specimens as shown in Table 9, representing HIV-1 Group M, subtypes A, B, C, D, F, G, H, J, K, CRF-A/E, CRF-A/G, CRF-B/C, group N and Group O. Each specimen was diluted to 3xLLoQ in K2 EDTA plasma and tested with one kit lot of the HIV-1 VL XC test. All samples tested at 3xLLoQ were reported as HIV-1 detected (Table 9).

Table 9. HIV-1 Specimens Tested at 3xLLoQ

HIV-1 Group	Subtype/CRF	Number of Samples Tested	Number of Samples Reported as HIV-1 Detected
M	A	10	10
	B	10	10
	C	10	10
	D	10	10
	F	10	10
	G	10	10
	H	10	10
	J	4	4
	K	8	8
	CRF-A/E	10	10
	CRF-A/G	11	11
	CRF-B/C	5	5
N	NA	1	1
O	NA	10	10

18.6 Analytical Specificity (Exclusivity)

The analytical specificity of the HIV-1 VL XC test was evaluated by adding potentially cross-reactive or interfering organisms at a concentration of 1 x 10^6 CFU/mL for microorganisms, or ≥ 1 x 10^5 copies/mL or $TCID_{50}$ for viruses into HIV-1 negative K2 EDTA plasma and K2 EDTA plasma containing HIV-1 reference material at a concentration of approximately 3xLLoQ. The HIV-1 reference material used was calibrated to the WHO 4th International standard for HIV-1 (NIBSC code: 16/194). Tested organisms are shown in Table 10. None of the tested organisms showed cross-reactivity or interfered with the quantification of the HIV-1 VL XC test.

Table 10. Analytical Specificity Organisms

Virus	Bacteria	Fungi/Yeast	Parasites
Chikungunya virus	*Mycobacterium tuberculosis*	*Candida Albicans*	Leishmania Major
Cytomegalovirus	*Propionibacterium acnes*	*Candida Glabrata*	Plasmodium Falciparum
Epstein-Barr virus	*Staphylococcus aureus*	*Candida Tropicalis*	Trypanosoma brucei
Hepatitis A virus	*Staphylococcus epidermidis*	Pneumocystis *jirovecii*	Trypanosoma cruzi
Hepatitis B virus	*Staphylococcus haemolyticus*		
Hepatitis C virus			
Herpes simplex virus 1			
Herpes simplex virus 2			
Human Herpesvirus 6			
Human Immunodeficiency virus 2			
Human T-cell lymphotropic virus type 1			
Human T-cell lymphotropic virus type 2			
Influenza virus A			

18.7 Potentially Interfering Substances

The susceptibility of the HIV-1 VL XC test to interference by elevated levels of endogenous substances, by drugs prescribed to HIV-1 infected patients or for those who may have co-infections or other co-morbidity, and autoimmune disease markers was evaluated. The inhibitory effects were evaluated in presence and absence of HIV-1 reference material at a concentration of approximately 3xLLoQ. The HIV-1 reference material used was calibrated to the WHO 4th International standard for HIV-1 (NIBSC code: 16/194).

Elevated levels of the endogenous substances shown in Table 11 were shown to not interfere with the quantification of the HIV-1 VL XC test or impact the specificity of the test when tested in presence and absence of HIV-1 RNA. All specimens tested in presence of HIV-1 RNA and the endogenous substance was quantified within ± 0.5 $\log_{10}$ copies/mL of the HIV-1 positive reference specimen. All specimens tested in absence of HIV-1 RNA were reported as HIV-1 Not Detected demonstrating that there was no impact on the specificity of the HIV-1 VL XC test.

Table 11. Endogenous Substances and Concentration Tested

Substance	Tested Concentration
Albumin	9 g/dL
Bilirubin	40 mg/dL
Hemoglobin	1000 mg/dL
Human DNA	0.4 mg/dL
Triglycerides	3000 mg/dL

The drug components as shown in Table 12 were shown to not interfere with the quantification or impact the specificity of the HIV-1 VL XC test when tested at three times peak level concentration (C_{max}) in the presence and absence of HIV-1 RNA.

Table 12. Drug Pools Tested

Pool	Drugs
1	Zidovudine, Clarithromycin, Interferon alfa-2b, Maraviroc, Rilpivirine, Ganciclovir
2	Abacavir sulfate, Peginterferon 2a, Ribavirin, Emtricitabine, Adefovir dipivoxil, Entecavir, Valganciclovir HCl
3	Tenofovir disoproxil fumarate, Lamivudine, 3TC, Raltegravir, Etravirine
4	Stavudine, d4T, Efavirenz, Lopinavir, Ciprofloxacin, Indinavir sulfate, Acyclovir
5	Nevirapine, Azithromycin, Telbivudine, Foscarnet[a], Cidofovir
6	Fosamprenavir calcium, Elvitegravir, Darunavir, Cobicistat, Atazanavir
7	Paritaprevir, Simeprevir
8	Daclatasvir, Elbasvir, Ledipasvir, Ombitasvir, Glecaprevir, Velpatasvir, Dasabuvir
9	Dolutegravir, Bictegravir, Doravirine, Maraviroc
10	Acetaminophen, Acetylsalicylic acid, Atorvastatin, Loratadine
11	Nadolol, Ascorbic acid, Phenylephrine, Ibuprofen
12	Artemether, Desethylamodiaquine, Mefloquine, Quinine
13	Primaquine, Chloroquine, Doxycycline
14	Rifampin, INH, Ethambutol, Pyrazinamide
15	Moxifloxacin, Levofloxacin, Amikacin, Bedaquiline[a]
16	Trimethoprim/Sulfamethoxazole, Gentamicin, Metronidazole, Ceftriaxone

[a] Tested individually instead of in combination with other drug components

Testing of K2 EDTA plasma specimens from five individuals positive for each of the autoimmune disease markers; systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA) or rheumatoid factor (RF) were shown to not interfere with the quantification of the HIV-1 VL XC test or impact the specificity of the test when tested in presence and absence of HIV-1 RNA.

18.8 Matrix Equivalency (K2 EDTA and PPT-EDTA)

Matrix equivalency for the HIV-1 VL XC test was conducted with matched clinical specimens from 50 HIV-1 positive individuals and 25 HIV-1 negative blood donors collected in K2 EDTA and PPT-EDTA collection tubes. The HIV-1 titers of the matched specimens (K2 EDTA and PPT-EDTA) from HIV-1 positive individuals covered the quantitative range of the test, 40-1x10^7 copies/mL.

Matrix equivalency for the HIV-1 VL XC test was demonstrated as shown in Figure 13. All HIV-1 positive specimens collected in PPT-EDTA media produced concentrations of HIV-1 RNA within ± 0.5 $\log_{10}$ copies/mL of the HIV-1 positive specimen collected in K2 EDTA media when tested with the HIV-1 VL XC test. All 25 matched HIV-1 negative specimens were reported as HIV-1 Not Detected.

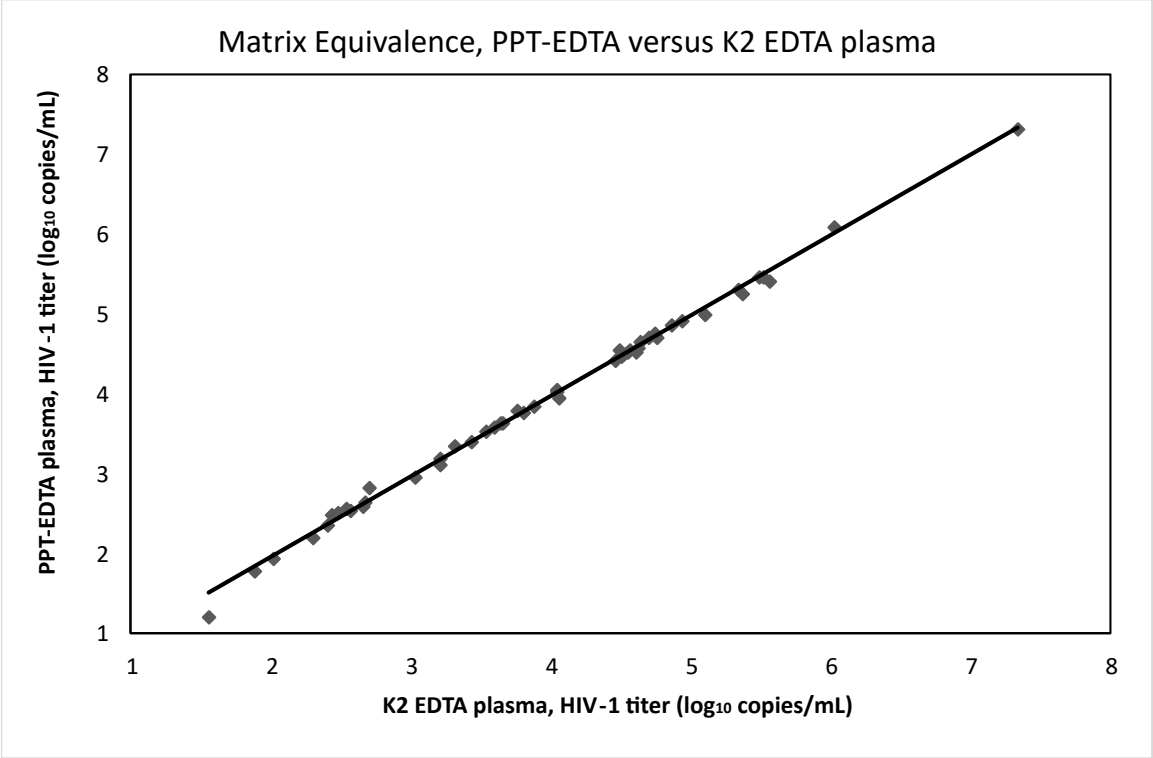


Figure 13. Linear Regression of HIV-1 titer (log_{10} copies/mL), PPT-EDTA plasma versus K2 EDTA plasma

18.9 Whole System Failure Rate

The whole system failure rate for the HIV-1 VL XC test was determined by testing 100 replicates of K2 EDTA plasma spiked with a HIV-1 subtype B specimen calibrated to the WHO 4th International Standard for HIV-1 (NIBSC code 16/194). The K2 EDTA plasma was spiked to a target concentration of 60 copies/mL and tested with one kit lot of the HIV-1 VL XC test.

The results of this study showed that all 100 replicates were valid and reported HIV-1 positive, resulting in a whole system failure rate of 0%.

18.10 Carry Over Contamination

A high titer HIV-1 positive specimen (>1 x 10^7 copies/mL) was tested, immediately followed by testing a HIV-1 negative specimen in the same GeneXpert instrument module. The procedure was repeated twenty (20) times in two different modules. The carryover rate for the HIV-1 VL XC test was 0%.

19 Performance Characteristics – Clinical Performance

19.1 Specificity

The specificity of the HIV-1 VL XC test was evaluated using 500 EDTA plasma specimens from HIV-1 negative blood donors. None of the 500 specimens tested were detected by the HIV-1 VL XC test equating to 100% specificity (95% CI = 99.2-100.0).

19.2 Method Correlation

A multi-site study was conducted to evaluate the performance of the HIV-1 VL XC test relative to a nucleic acid amplification test (NAAT) comparator method using fresh and frozen human plasma specimens collected from known HIV-1 infected individuals. Of the 362 specimens, each from unique individuals, 206 (56.9%) were collected from male subjects. Most individuals (94.5%; 342/362) were in the age range of 22 to 59 years. Classification of specimens by HIV-1 Group M subtypes in this study population were shown to be 25.1% subtype B, 16.1% non-B subtype and 58.8% subtype unknown.

There were 21 indeterminate results of which 14 were resolved after retesting. The final indeterminate rate was 1.93% (7/362).

Of the 362 specimens, 328 were within the quantitation range of Xpert HIV-1 VL XC and the comparator test. The Deming regression shows high correlation between the Xpert HIV-1 VL XC test and the comparator method with a slope of 0.9625 and intercept of 0.0198. The R2 was 0.9561.

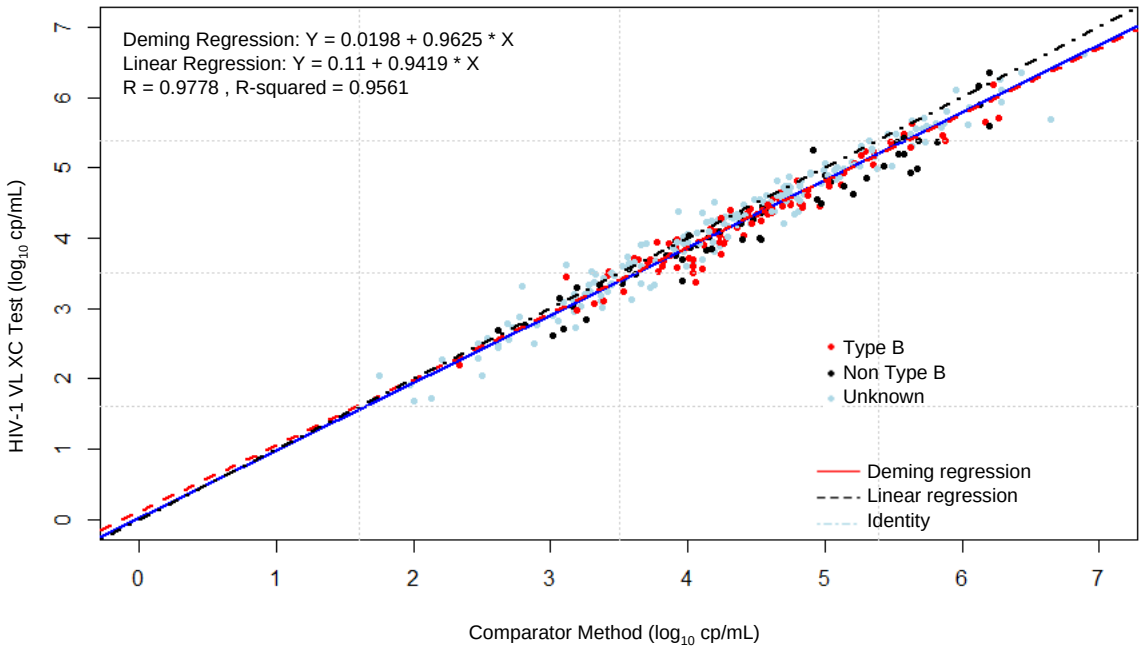


Figure 14. Correlation between the HIV-1 VL XC Test Relative to a Comparator Method

20 References

1. Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996; 272:1167–1170.
2. World Health Organization. What's new in Treatment Monitoring: Viral Load and CD4 Testing. Geneva. WHO. 2017
3. WHO International Standard; 4th HIV-1 International Standard (NIBSC code: 16/1094). National Institute for Biological Standards and Control; 2017.
4. 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.
5. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
6. World Health Organization. Safe management of wastes from health-care activities. 2nd Edition. WHO, 2014. Accessed July 24, 2020 at http://www.who.int/water_sanitation_health/publications/wastemanag/en/
7. 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, and amending Regulation (EC) No 1907/2006.
8. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).
9. Clinical and Laboratory Standards Institute. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI document EP17-A2. Wayne, PA, 2012.

10. Clinical and Laboratory Standards Institute. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA, 2014.

11. Clinical and Laboratory Standards Institute. Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach. Approved Guideline. CLSI document EP06-A. Wayne, PA, 2003.

21 Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

22 Technical Assistance

Before Contacting Us

Collect the following information before contacting Cepheid Technical Support:

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

Report serious incidents associated with the test to Cepheid and the competent authority of the member state in which the serious incident occurred.

United States

Telephone: + 1 888 838 3222
Email:
techsupport@cepheid.com

France

Telephone:+ 33 563 825 319
Email:
support@cepheideurope.com

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

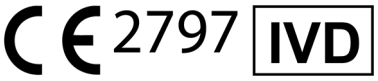
23 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	CE marking – European Conformity
	Do not reuse
	Batch code
	Consult instructions for use
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Caution
	Warning
	Authorized Representative in Switzerland
	Importer

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
CH-8800
Switzerland

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
CH-8800
Switzerland



24 Revision History

Description of Changes: From 302-4124 Rev. C to Rev. D

Purpose: Addition of Swiss Importer details.

Section	Description of Change
23	Addition of Swiss Importer symbol, CH REP symbol, and related addresses.