WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: Xpert HIV-1 Viral Load with GeneXpert Dx, GeneXpert Infinity-48s, GeneXpert Infinity-80 and GeneXpert Edge System
WHO reference numbers: PQDx 0192-070-00[1], PQDx 0193-070-00, PQDx 0194-070-00, PQDx 0195-070-00

Xpert HIV-1 Viral Load with **GeneXpert Dx, GeneXpert Infinity-48s, GeneXpert Infinity-80[2], and GeneXpert Edge** product code **GXHIV-VL-CE-10**, manufactured by **Cepheid AB**, **CE marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 20 July 2017.

Summary of WHO prequalification assessment for Xpert HIV-1 Viral Load

	Date	Outcome
PQ listing	20 July 2017	listed
Dossier review	N/A	MR
Site inspection(s) of the quality management system	16-24 March 2022 8-10 November 2022	MR
Product performance evaluation	19 June 2017	MR

MR: Meets requirements
N/A: Not applicable

Report amendments and product changes
This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which the WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

[1] Please note that Gene Xpert 48 is now obsolete and removed from this report.
[2] Please note that other configurations of GeneXpert system such as GeneXpert I, GeneXpert II, GeneXpert IV, GeneXpert XVI are covered by this prequalification assessment, see section on instrumentation.

Version	Summary of amendment	Date of report amendment
2.0	Update on instructions for use due to the improved product stability, robustness and quantification of HIV-1 rare polymorphisms in group M (first observed in subtype C included in the QCMD panel).	13 November 2017
3.0	Addition of a defined intended end user and updated instructions for use.	21 March 2018
4.0	Cepheid's notified body changed from LRQA (UK) to BSI (Netherlands). Labels and IFUs were updated accordingly.	6 June 2019
5.0	Extention of the shelf-life from 12 months to 18 months. Addition of a new GeneXpert DX instrument product code for use with all prequalified assays. New GeneXpert DX instrument models make use of GeneXpert modules which have a 10-channel optical system modules, compared to the 6-channel optical system that pre-existed.	3 December 2020
6.0	Correction of omitted application number, PQDx 0193-070-00.	24 August 2021
7.0	Revision of IFU to add instructions for using the GeneXpert Edge System, which was previously approved for use with the Xpert HIV-1 Qual test (PQDx 0259-070-00). The change calls for listing the new part number for the Edge instrument, GXI-EDGE-L, and the Edge Software 1.0 in the public report. Addition of information regarding the most recent onsite inspections of the manufacturing facility.	15 May 2023

Intended use:

According to the claim of Cepheid AB, "*the Xpert HIV-1 VL assay is an in vitro reverse transcriptase polymerase chain reaction (RT PCR) assay for the detection and quantification of Human Immunodeficiency Virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals, using the automated GeneXpert Instrument Systems. The assay can quantify HIV-1 RNA over the range of 40 to 10,000,000 copies/mL. The Xpert* HIV-*1 VL assay 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 assay 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. The assay is intended to be used by laboratory professionals or specifically-trained healthcare workers.

The Xpert HIV-1 VL assay is not intended to be used as a blood donor screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection".

Assay Description:

According to the claim of Cepheid AB, "*GeneXpert Instrument Systems automate and integrate specimen preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex specimens using real-time reverse transcriptase PCR (RT-PCR). The systems consist of an instrument, a personal computer with preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the RT-PCR reagents and host the RT-PCR processes. Because the cartridges are self-contained, cross-contamination between specimens is minimized.*

For a full description of the systems, refer to the appropriate GeneXpert Dx Operator Manual or GeneXpert Infinity Operator Manual.

Xpert HIV-1 Viral Load includes reagents for the detection of HIV-1 RNA in specimens 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. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability".

Test kit contents:

Xpert HIV-1 Viral Load	**10 tests (Product code GXHIV-VL-CE-10)**
Xpert HIV-1 Viral Load cartridges with integrated reaction tubes	10
Disposable 1 mL transfer pipettes	1 bag of 10 per kit
CD (includes instructions for use)	1

Instrumentation:

Product name	**Product code(s)**
GeneXpert Dx (including barcode scanner and operator manual)	GXI-1-L, GXI-1-D, GXII-1-L, GXII-1-D, GXII-2-L, GXII-2-D, GXIV-1-L, GXIV-1-D, GXIV-2-L, GXIV-2-D, GXIV-3-L, GXIV-3-D, GXIV-4-L, GXIV-4-D, GXXVI-4-L, GXXVI-4-D, GXXVI-8-L, GXXVI-8-D, GXXVI-12-L, GXXVI-12-D, GXXVI-16-L, GXXVI-16-D, GXI-1-D-10C GXIV-4-D-10C GXI-1-L-10C, GXIV-4-L-10C, GXII-1-D-10C GXXVI-4-D-10C, GXII-1-L-10C GXXVI-4-L-10C

	GXII-2-D-10C, GXXVI-8-D-10C, GXII-2-L-10C GXXVI-8-L-10C, GXIV-1-D-10C GXXVI-12-D-10C, GXIV-1-L-10C, GXXVI-12-L-10C, GXIV-2-D-10C, GXXVI-16-D-10C, GXIV-2-L-10C, GXXVI-16-L-10C, GXIV-3-D-10C, and GXIV-3-L-10C
GeneXpert Infinity-48s (including barcode scanner and operator manual)	INFINITY48-16, INFINITY48-16-EUROPE, INFINITY48-24, INFINITY48-24-EUROPE, INFINITY48-32, INFINITY48-32-EUROPE, INFINITY48-40, INFINITY48-40-EUROPE, INFINITY48-48, INFINITY48-48-EUROPE
GeneXpert Infinity-80 (including barcode scanner and operator manual)	INFINITY80-16, INFINITY80-16-230V, INFINITY80-24, INFINITY80-24-230V, INFINITY80-32, INFINITY80-32-230V, INFINITY80-40, INFINITY80-40-230V, INFINITY80-48, INFINITY80-48-230V, INFINITY80-56, INFINITY80-56-230V, INFINITY80-64, INFINITY80-64-230V, INFINITY80-72, INFINITY80-72-230V, INFINITY80-80, INFINITY80-80-230V
GeneXpert Dx Software Version 4.6a or higher (GeneXpert Dx systems), Xpertise 4.6 or higher, or Xpertise 6.2 or higher (Infinity-80/Infinity-48s)	GX4.0SWKIT, XPERTISE-G2-SWKIT
GeneXpert Edge instrument and the Edge Software 1.0	GXI-EDGE-L

Items required but not provided:

Item
Consumables: Bleach 70% Ethanol Disposable gloves EDTA specimen tubes EDTA plasma preparation tubes ACD specimen tubes
Equipment: Printer Centrifuge for processing serum and plasma specimens

Storage:
The test kit (Xpert HIV-1 Viral Load cartridges) should be stored at 2 °C -28 °C.

Shelf-life upon manufacture:
18 months.

Warnings/limitations:
Xpert HIV-1 Viral Load 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.

Specimen preparation: Whole blood may be held at 15–30 °C for up to 8 hours, 15–25 °C for up to 24 hours or at 2–8 °C for up to 3 days prior to preparing and testing the specimen. After centrifugation, plasma may be held at 15–30 °C for up to 24 hours or at 2–8 °C for up to 6 days before testing. **Plasma must be removed from the primary collection tube after centrifugation for storage.**

This product contains Guanidinium Thiocyanate (GTC). According to the European Chemicals Agency, this substance is considered corrosive and a health hazard. It can cause severe skin burns and eye damage, is harmful if swallowed, is harmful if inhaled, is harmful in contact with skin and is harmful to aquatic life with long-lasting effects. Users should therefore be aware of first aid measures and special measures for safe disposal. The manufacturer has information on safe use, first aid measures and disposal. It is recommended that a copy of this information is requested[3].

Prioritization for prequalification
Based on the established eligibility criteria, Xpert HIV-1 Viral Load was given priority for the WHO prequalification assessment.

Product dossier assessment

In accordance with the WHO procedure for abbreviated prequalification assessment, Cepheid AB was not required to submit a product dossier for Xpert HIV-1 Viral Load as per the "*Instructions for compilation of a product dossier*" (PQDx_018 v1). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

[3] *For Use in Low and Middle-Income Countries: Disposal of Xpert Assay Components Containing GTC*

Commitment for prequalification:

The manufacturer will make the "For Use in Low and Middle-Income Countries: Disposal of Xpert Assay Components Containing GTC" available with every new consignment of GeneXpert instruments and ensure all current end-users are aware of disposal and safety measures.

The end user assessment submitted by Cepheid addressing the above-mentioned nonconformity was found acceptable in meeting WHO prequalification requirements on 14 December 2017.

Manufacturing site inspection

In accordance with the WHO procedure for abbreviated prequalification assessment, an inspection was conducted at the following sites of manufacture

- Röntgenvägen 5, SE-171 54 Solna (Stockholm), Sweden, between 16 and 24 March 2022 where Xpert HIV-1 Viral Load with GeneXpert Dx, GeneXpert Infinity-48s, GeneXpert Infinity-80 and GeneXpert Edge System were reviewed; and
- 1631 220th Street SE, Bothel, WA, 98021, USA, between 8 and 10 November 2022, where GeneXpert Dx, GeneXpert Infinity-48s, GeneXpert Infinity-80 and GeneXpert Edge System were reviewed;
  as per the "Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics" (PQDx_014 v1).

The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 25 October 2022 and 8 May 2023.

Based on the site inspection and corrective action plan review, the quality management system for Xpert HIV-1 Viral Load with GeneXpert Dx, GeneXpert Infinity-48s, GeneXpert Infinity-80, and GeneXpert Edge System meets WHO prequalification requirements.

To note:

Due to the COVID-19 pandemic and the corresponding national and international travel restrictions, some inspection dates may be impacted.

Please see below the link to the official statement of the PQ team regarding the impact of COVID-19 on Prequalification activities:
https://www.who.int/diagnostics_laboratory/eual/impact_covid-19_PQT/en/

Product performance evaluation

Xpert HIV-1 Viral Load was evaluated from 18 January 2016 to 21 April 2016 and from 20 December 2016 to 11 May 2017 at the NHLS HIV PCR Laboratory, Charlotte Maxeke Johannesburg Academic Hospital, South Africa and the International Laboratory Branch, Division of Global HIV and TB, CDC Atlanta. From this evaluation, we drew the following conclusions.

Xpert HIV-1 Viral Load is a cartridge-based, total nucleic acid real-time RT-PCR assay for monitoring HIV-1 viral load in human plasma specimens. A volume of 1000 µl of plasma specimen is needed to perform the assay (if using the transfer pipette included in the kit, a minimum of 1.2 mL of plasma is required). This type of assay requires additional laboratory equipment for specimen preparation, including a centrifuge and refrigerator (if the storage of specimens is needed) but can be performed in laboratories with limited facilities. The instrument requires a stable source of electricity.

Analytical specimens

The assay's precision of measurement was verified. In this evaluation, the precision of measurement was found to be acceptable; all %CVs were found to be < 3%.

The linearity of the assay was verified in Subtypes A, B, C, D, and AG. In this evaluation the linearity for all subtypes were estimated by linear regression. All slopes were < 5% from an ideal value of 1. R^2 values were all > 0.99 indicating good linearity over a range of viral load values of 10^3 to 10^6 copies/ml.

The limit of detection was verified. In this evaluation, the LOD was estimated to be 38 IU/ml (95% Fiducial limits: 27-139); 22 copies/ml (95% Fiducial limits: 16-80).

No carry-over was detected.

Clinical specimens

In this performance evaluation on a panel of 439 specimens, we found a bias of 0.043 log10 copies/ml [95% CI (-0.986; 1.073)] compared to the reference results (Roche COBAS Ampliprep/COBAS TaqMan HIV-1 (Roche CAP/CTM HIV-1 Test, v2.0)).

The correlation between the assay under evaluation and the reference results was within range (R^2 =0.941, P<0.001).

The sensitivity for virological failure at 1000 copies/ml was 94.14% (95%CI: 90.37-96.76).
The specificity for virological failure at 1000 copies/ml was 98.50% (95% CI: 95.68-99.69).

The overall invalid rate calculated with data from both sites was 2.94%.

Performance characteristics	
Analytical performance	
Limit of Detection	38 IU/ml (95% Fiducial limits: 27-139); 22 copies/ml (95% Fiducial limits: 16-80)
Linearity	Verified in subtypes: A, B, C, D, and AG Linearity for all subtypes was determined to be acceptable. All slopes were within 5% of 1, and R^2 > 0.99.
Carry-over	0%
Clinical performance	
Bias	0.043 log10 copies/ml [95% CI (-0.986; 1.073)]
Correlation	R^2 =0.941, P<0.001
Sensitivity for virological failure at 1000 copies/ml (N=239)	94.14% (95%CI: 90.37-96.76).
Specificity for virological failure at 1000 copies/ml (N=200)	98.50% (95% CI: 95.68-99.69).
Invalid rate	2.94%

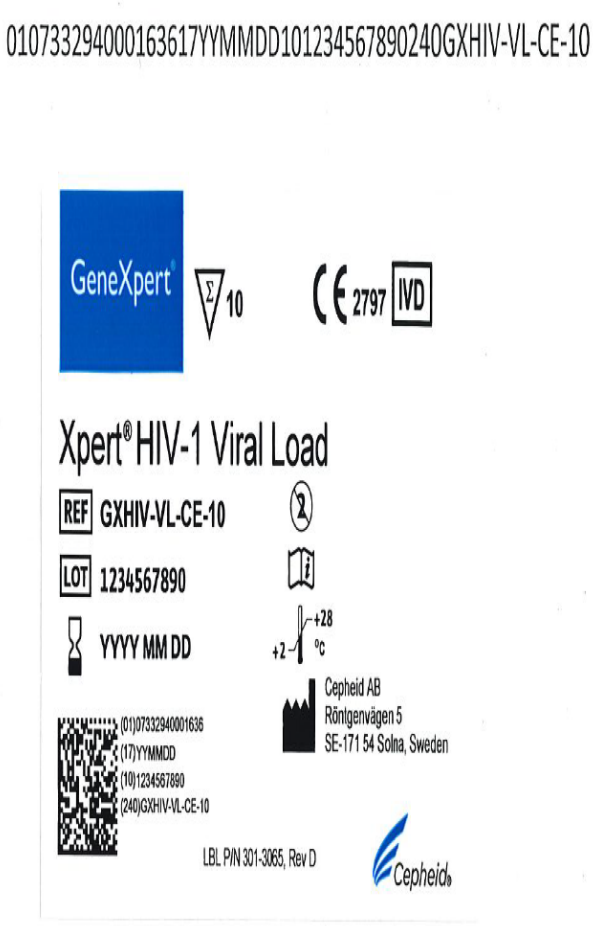
Key operational characteristics	
Validated specimen types	EDTA plasma, EDTA plasma collected in EDTA plasma preparation tubes (aliquoted immediately after separation).
Number of steps	4 from the addition of the specimen to the result.
Time to result	1h:33 minutes (preparation and loading: 3 minutes; test: 90 minutes).
In-use stability of reagents	Reagents are all contained within the cartridge.

Labelling

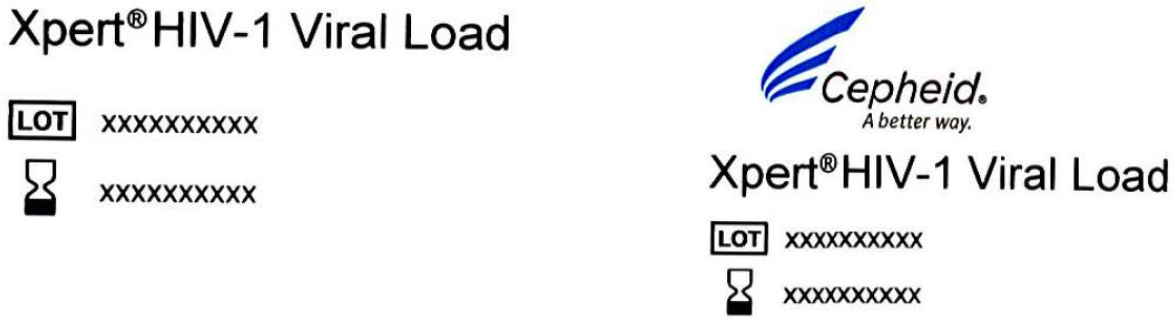
1. **Labels**
2. **Instructions for use**

1.0 Labels

Kit carton label

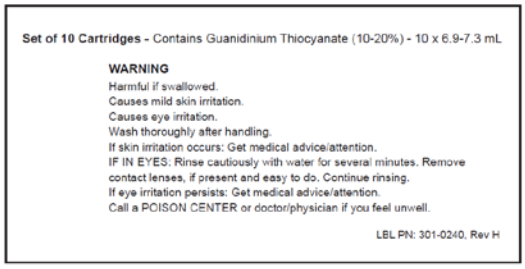


Cartridge label



LBL P/N 301-3395, Rev B

HCV VL, HIV Qual and HIV VL 10-Test Kits
Set of 10 Cartridges - Guanidinium Thiocyanate (10-20%) - 6.9-7.3 mL
Hazard Warning Label
Updated 04/20/18



Stock Label p/n 301-0240, Rev H

Material:	Transtherm 1C Paper
Colors:	Black
Adhesive:	AT20
Topcoat:	Full UV Varnish
Label Size:	4.0" x 2.0"
Copy Unwind Position:	#4
General Specifications:	Reference Cepheid Doc D7280 for additional requirements

2.0 Instructions for use[4]

[4] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® HIV-1 Viral Load

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

REF **GXHIV-VL-IN-10**

Instructions for Use

CE 2797 **IVD**

Cepheid®

In Vitro Diagnostic Medical Device

301-3068, Rev. M
December 2021

Trademark, Patents and Copyright Statements

See Section 26 Revision History for a description of changes.

Xpert® HIV-1 Viral Load

For In Vitro Diagnostic Use Only.

1 Proprietary Name

Xpert® HIV-1 Viral Load

2 Common or Usual Name

HIV-1 VL

3 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 HIV-1 infected individuals, 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 assay 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. 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.

4 Summary and Explanation

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS).[1,2,3] 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.[4,5,6]

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.[7,8,9]

HIV diagnostics have evolved significantly in the past two decades and continue to be important for managing the treatment and care of HIV infected patients. Measurement of blood plasma HIV-1 RNA concentration or viral load using nucleic acid-based molecular diagnostic tests 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.[10,11,12,13,14,15]

The Xpert HIV-1 VL test uses reverse transcriptase 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 specimens using real-time reverse transcriptase PCR (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 Infinity System Operator Manual*, or *GeneXpert Edge System User's Guide*.

The Xpert HIV-1 VL test includes reagents for the detection of HIV-1 RNA in specimens 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. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert HIV-1 VL test kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert HIV-1 VL Test 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 Xpert HIV-1 VL test cartridges at 2–28 °C. Prior to use, bring the cartridges to room temperature.
- Do not open the cartridge lid until you are ready to perform the test.
- Use cartridge within four hours after opening the cartridge lid.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

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

9 Warnings and Precautions

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[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.
- 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 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 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.
- 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 disposal. If country 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*.

10 Chemical Hazards[18,19]

- 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 EYE: 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 Specimen 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 either EDTA or ACD 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 assay. If using the transfer pipette included in the kit, a minimum of 1.2 mL plasma is required (see instructions in Preparing the Cartridge, Option 1 below). Alternatively, if using a precision pipette, a minimum of 1 mL plasma is required.
- Whole blood collected BD Vacutainer PPT Plasma Preparation Tubes for Molecular Diagnostic Test Methods, or in sterile collection tubes using either EDTA or ACD as the anticoagulant may be held at 15–30 °C for up to 8 hours, 15–25 °C for up to 24 hours or at 2–8 °C for up to 72 hours, prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Plasma separated from whole blood may be held at 15–30 °C for up to 24 hours, at 2–8 °C for up to 6 days or frozen(≤ -18 °C and ≤ -70 °C) for up to 6 weeks prior to testing. Plasma should be removed from the primary collection tube after centrifugation for storage.
- Plasma specimens are stable for up to three freeze/thaw cycles.

12 Procedure

12.1 Preparing the Specimen

1. Following centrifugation of whole blood specimens, 1 mL of plasma may be pipetted directly into the test cartridge. Sufficient volume is critical to obtaining valid test results (see instructions in Preparing the Cartridge, Option 1 below).
2. Frozen plasma specimens should be completely thawed and equilibrated to room temperature (20–35 °C) prior to testing.
3. Plasma specimens stored at 2–8 °C should be removed from the refrigerator and equilibrated to room temperature (20–35°C) prior to testing.
4. Plasma specimens stored at 2–8 °C or frozen and thawed should be vortexed for 15 seconds before use. If the specimen is cloudy, clarify by a quick (10 second) centrifugation.

12.2 Preparing the Cartridge

Important **Start the test within four hours of adding the specimen to the cartridge.**

Note

Pipetting less than 1 mL of plasma into the cartridge will trigger an insufficient volume error (ERROR 2097), preventing the instrument from running the sample (see Option 1 below).

Allow Xpert HIV-1 VL test cartridges and specimens to come to room temperature prior to pipetting plasma into the cartridge.

1. Wear protective disposable gloves.
2. Inspect the test cartridge for damage. If damaged, do not use it.
3. Open the lid of the test cartridge.

Note There is a thin plastic film that covers the inner ring of 13 ports of the test cartridge. This film should not be removed.

- **Option 1:** If using the transfer pipette included in the kit (Figure 1), fill to just below the bulb but above the line to transfer at least 1 mL plasma from the collection tube into the sample chamber of the test cartridge (Figure 2). Do **NOT** pour the specimen into the chamber!
- **Option 2**: If using an automatic pipette, transfer at least 1 mL of plasma into the sample chamber of the test cartridge (Figure 2). Do **NOT** pour the specimen into the chamber!

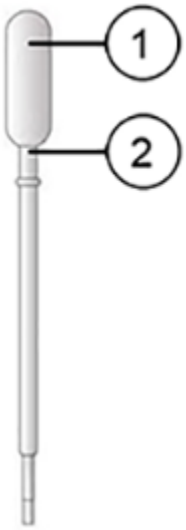


Figure 1. Xpert HIV-1 VL Test Transfer Pipette

Number	Description
1	Bulb
2	Fill specimen to just below the bulb and above the mark on the pipette.

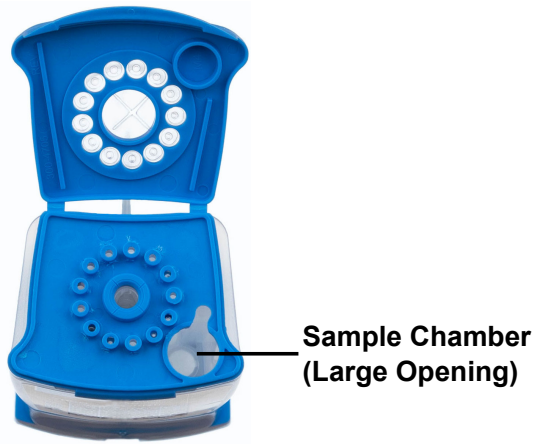


Figure 2. Xpert HIV-1 VL Cartridge (Top View)

4. Close the cartridge lid, and start the test:
   - For the GeneXpert Dx System, see Section 13.
   - For the GeneXpert Edge System, see Section 14.
   - For the GeneXpert Infinity System, see Section 15.

13 GeneXpert Dx System

13.1 Starting the Test

Important **Before you start the test, make sure that the system is running GeneXpert Dx software version 4.7b or higher and that 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.

13.2 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.

14 GeneXpert Edge System

(May not be available in all countries)

14.1 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.

14.2 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.

15 GeneXpert Infinity System

15.1 Starting the Test

Important **Before you start the test, make sure that the system is running Xpertise software version 6.4b or higher and that 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 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. After the cartridge is scanned, the **Order Test - Test Information** workspace displays.

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.

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.

15.2 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. Chick the **REPORT** button to view and/or generate a PDF report file.

16 Quality Control

Each test includes a Sample Volume Adequacy (SVA) control, Internal Quantitative Standard High and Low (IQS-H and IQS-L), which is also a sample processing control, 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 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.
- **Internal Quantitative Standard High and Low (IQS-H and IQS-L)**: IQS-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 IQS-L 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.
- **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.
- **External Controls**: Following good laboratory practice, external controls, not available in the kit, should be used in accordance with the requirements of local and state accrediting organizations as applicable.

17 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 and Figure 5). Possible results are shown in Table 1.

Table 1. HIV-1 VL Results and Interpretation

Result	Interpretation
HIV-1 DETECTED **XX copies/mL** See Figure 3 and Figure 4.	HIV-1 RNA is detected at XX copies/mL. ● HIV-1 RNA has quantitative value within the analytical measurement range. ● IQS-H and IQS-L: PASS. ● Probe Check: PASS; all probe check results pass.
HIV-1 DETECTED **> 1 × 10 ^{7} copies/mL**	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**	HIV-1 RNA is detected below the analytical measurement range. ● IQS-H and IQS-L: PASS. ● Probe Check: PASS; all probe check results pass.

Result	Interpretation
HIV-1 NOT DETECTED See Figure 5 and Figure 6.	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	Presence or absence of HIV-1 RNA cannot be determined. Repeat test according to the instructions in Retest Procedure. • IQS-H and/or IQS-L: FAIL; Cycle thresholds (Cts) are not within valid range. • Probe Check: PASS; all probe check results pass.
ERROR	Presence or absence of HIV-1 RNA cannot be determined. Repeat test according to the instructions in Retest Procedure. • Probe Check: FAIL; all or one of the probe check results fail.
NO RESULT **NO RESULT - REPEAT TEST**[a] See Figure 7.	Presence or absence of HIV-1 RNA cannot be determined. Repeat test according to the instructions in Retest Procedure. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

[a] For GeneXpert Edge only

Note Results can be converted from copies/mL to IU/mL within the software. See the appropriate *GeneXpert Dx System Operator Manual*, *GeneXpert Infinity System Operator Manual*, or *GeneXpert Edge System User's Guide* for instructions on how to change this setting. The conversion factor for the Xpert HIV-1 VL test is 1 copy = 1.72 International Unit (IU).

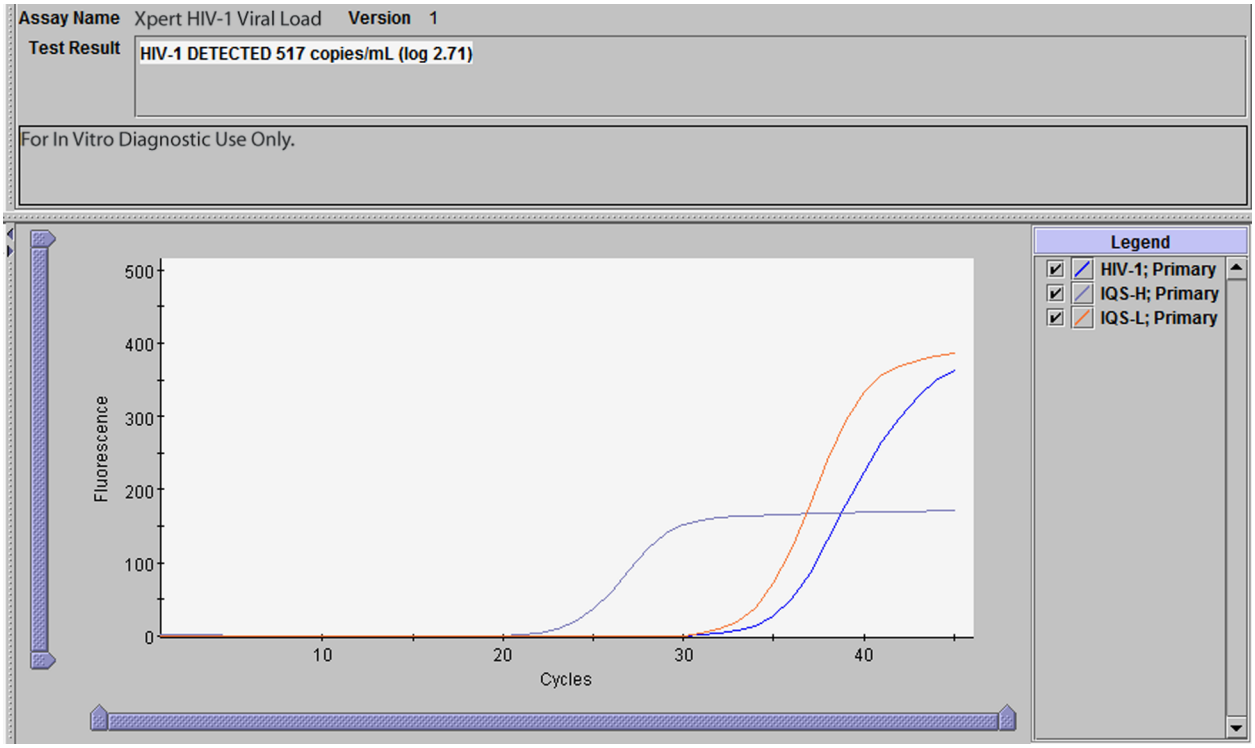


Figure 3. HIV-1 Detected as displayed in the GeneXpert Dx System and GeneXpert Infinity System

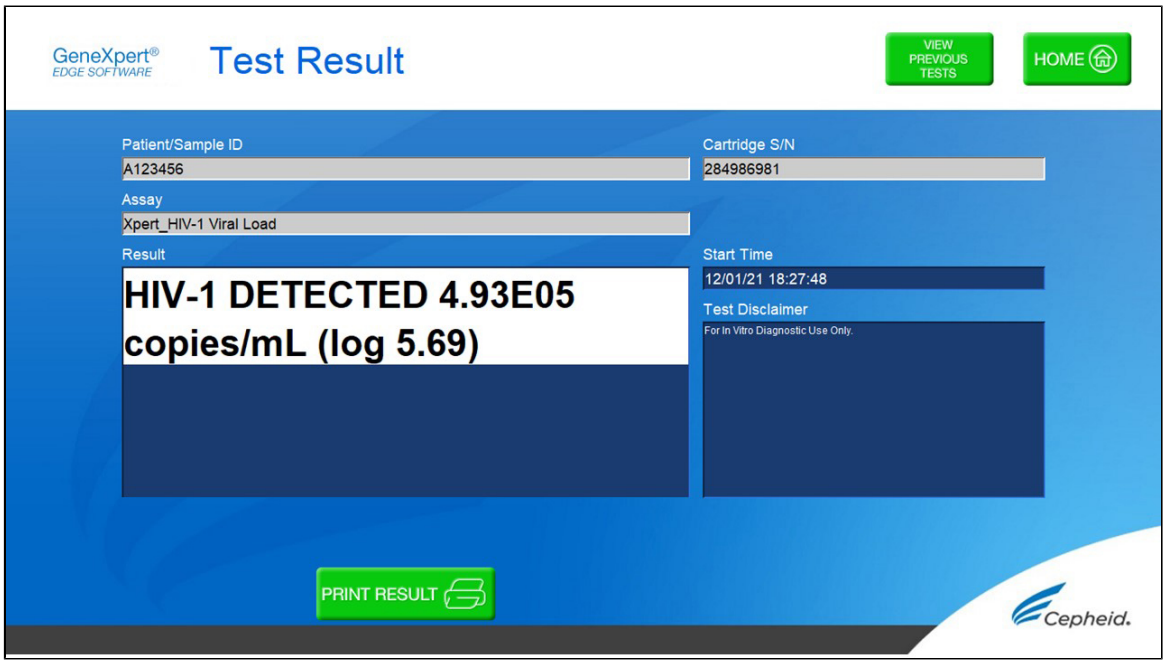


Figure 4. HIV-1 Detected as displayed in the GeneXpert Edge System

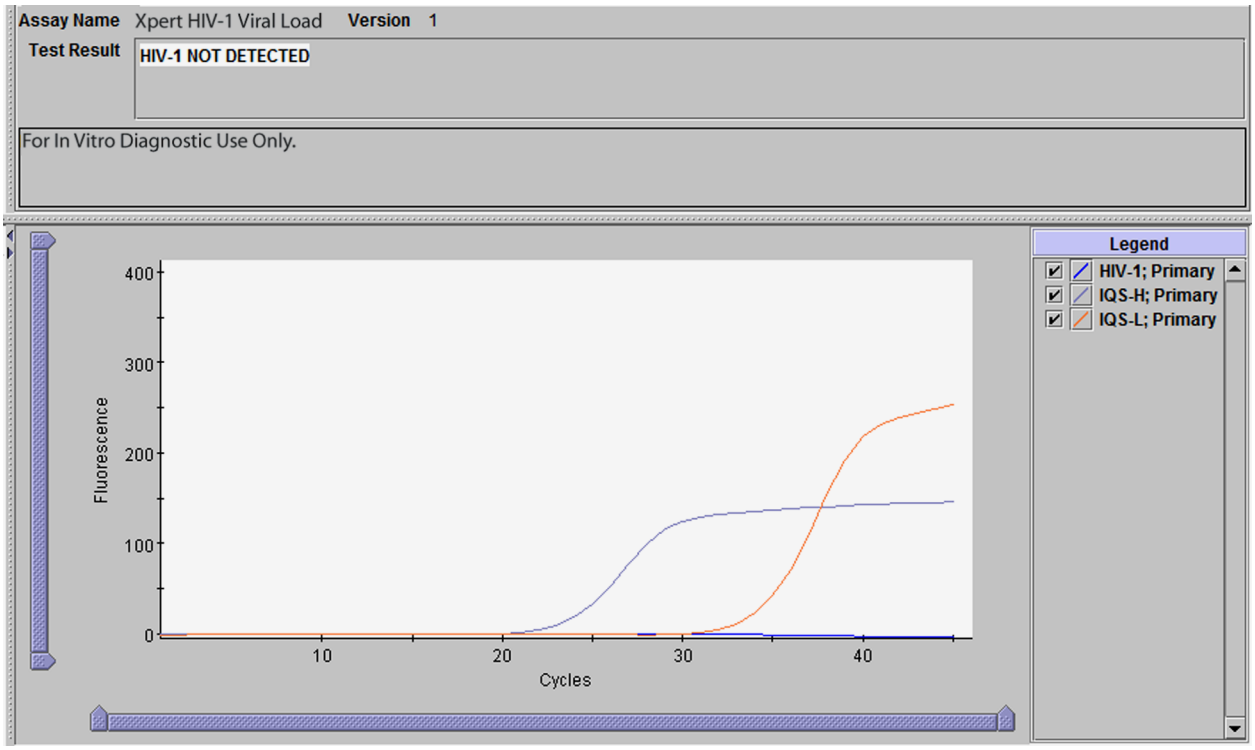


Figure 5. HIV-1 Not Detected as displayed in the GeneXpert Dx System and GeneXpert Infinity System

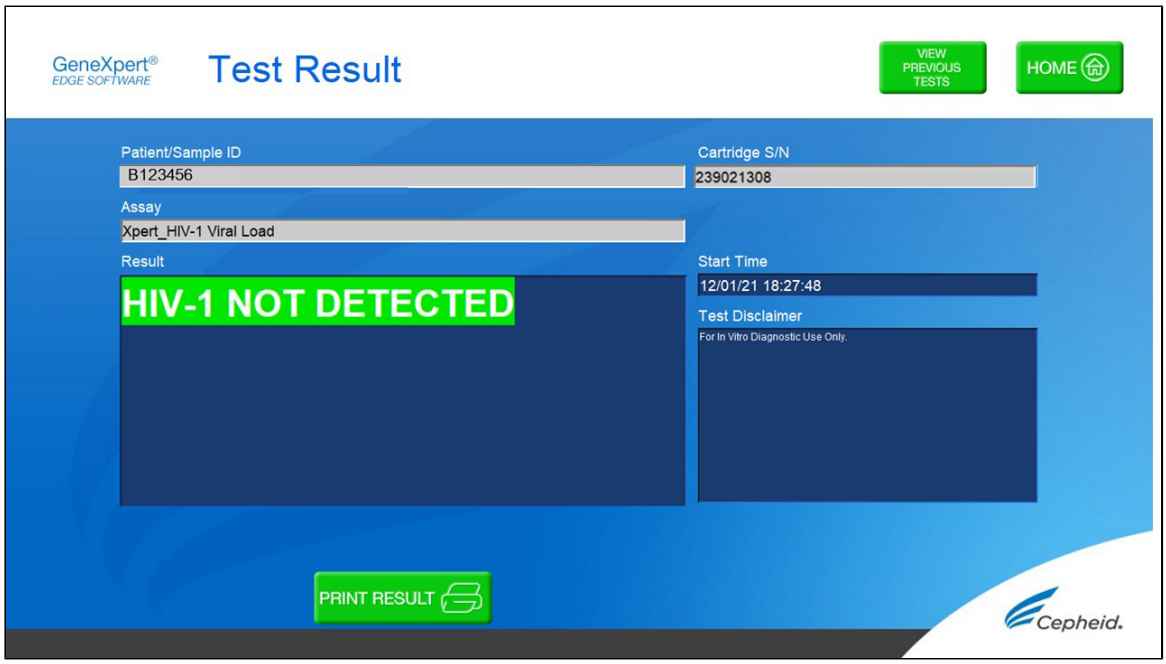


Figure 6. HIV-1 Not Detected as displayed in the GeneXpert Edge System

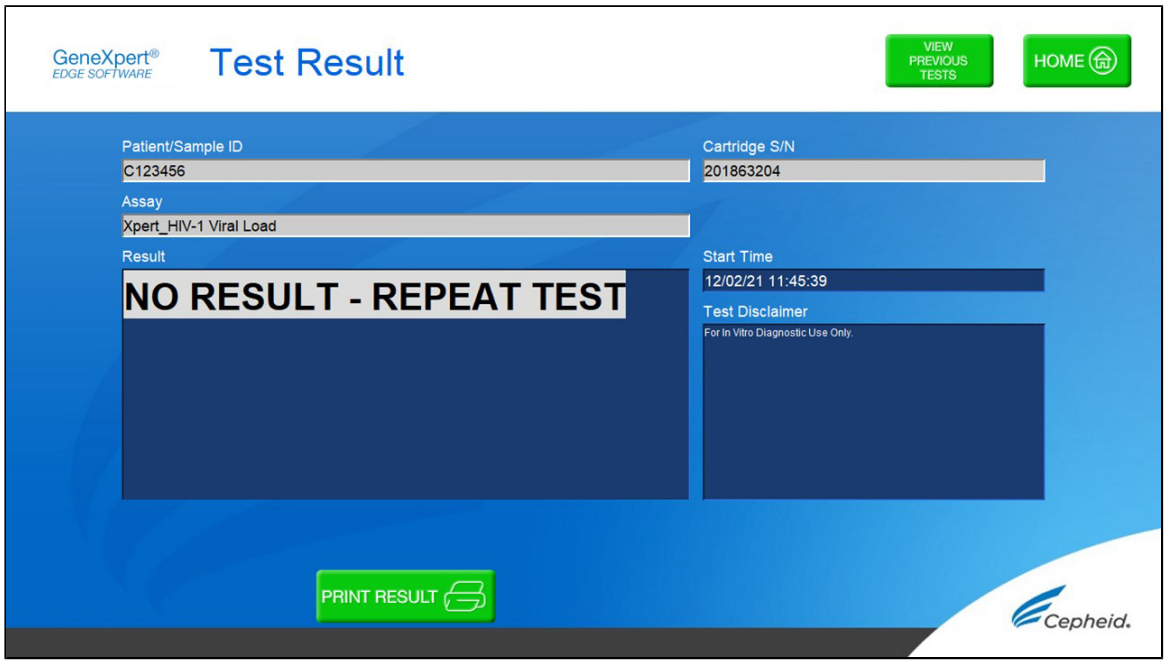


Figure 7. No Result - Repeat Test as displayed in the GeneXpert Edge System

18 Retests

18.1 Reasons to Repeat the Test

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

- 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** result 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.

18.2 Retest Procedure

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

1. Remove a new cartridge from the kit.
2. Start another test:
   - For the GeneXpert Dx System, see Section 13.
   - For the GeneXpert Edge System, see Section 14.
   - For the GeneXpert Infinity System, see Section 15.
3. A specimen that yields **INVALID** results twice is likely to contain an inhibitor; retesting is not recommended.

19 Performance Characteristics

19.1 Limit of Detection

The limit of detection (LOD) of the Xpert HIV-1 VL test was determined by testing five different dilutions prepared from two different HIV-1 subtype B reference standards, one cell culture stock, and two clinical specimens diluted in HIV-1 negative EDTA plasma. The HIV-1 subtype B materials used in the LOD study included Viral Quality Assurance Laboratory (VQA) reference material from the AIDS Clinical Trials Group, WHO 3rd HIV-1 International Standard (NIBSC code: 10/152), cell culture stock isolate BK132 and two clinical specimens. The assignment of the nominal concentration of the cell culture stock material and clinical specimens was performed by the Abbott RealTi*m*e HIV-1 Assay. The limit of detection was determined for three kit lots and a total of 72 replicates per level. The evaluation was performed according to CLSI guideline E17-A2.[20] The HIV-1 RNA concentration that can be detected with a positivity rate of greater than 95% was determined by Probit regression analysis. The results for the individual lots and specimens are shown in Table 2. The maximum/highest observed LOD with WHO reference standard for HIV-1 subtype B in EDTA plasma was 21.1 copies/mL (95% CI 16.1-26.0). The maximum/highest observed LOD with VQA reference standard for HIV-1 subtype B in EDTA plasma was 16.3 copies/mL (95% CI 13.0-19.5).

Table 2. Xpert HIV-1 VL Test LOD Estimates with Probit Regression and 95% Upper and Lower Confidence Intervals for HIV-1 Subtype B Specimens in EDTA Plasma

Specimen	Lot	LOD (copies/mL)	95% CI
WHO	Lot 1	21.1	16.1–26.0
	Lot 2	14.3	11.2–17.5
	Lot 3	19.0	14.3–23.7
VQA	Lot 1	15.5	12.5–18.6
	Lot 2	14.0	11.2–16.7
	Lot 3	16.3	13.0–19.5
Clinical Specimen 1	Lot 1	24.0	18.1–29.9
	Lot 2	25.5	19.5–31.5
	Lot 3	23.1	17.5–28.7

Specimen	Lot	LOD (copies/mL)	95% CI
Clinical Specimen 2	Lot 1	20.3	15.8–24.7
	Lot 2	15.4	12.0–18.7
	Lot 3	28.5	21.3–35.7
Cell Culture Specimen	Lot 1	18.8	14.6–23.1
	Lot 2	20.0	15.6–24.4
	Lot 3	32.0	24.7–39.3

The LOD for the VQA reference material was also confirmed in ACD plasma using one reagent lot. The LOD estimate for the HIV-1 subtype B VQA specimen in ACD plasma was 15.8 copies/mL (95% CI 12.1-19.5).

The LOD for the HIV-1 subtype B in EDTA plasma was evaluated with two different sets of standards and three kit lots of the Xpert HIV-1 VL test using Probit Analysis:

- LOD with WHO 3rd International Standard: 18.3 copies/mL (95% CI 15.9-20.8)
- LOD with VQA reference material: 15.3 copies/mL (95% CI 13.5-17.0)

Hit rate analysis shows a positivity rate of >95% at 40 copies/mL for all for HIV-1 subtype B materials tested as shown in Table 3. The LOD for the Xpert HIV-1 VL test is determined to be 40 copies/mL for HIV-1 subtype B in EDTA and ACD plasma.

Table 3. Xpert HIV-1 VL Test LOD for HIV-1 Subtype B Specimens in EDTA Plasma

Specimen	Nominal concentration (copies/mL)	No. Replicates	No. Positives	Positivity Rate (%)
WHO	1	72	10	14
	2.5	72	18	25
	5	72	40	56
	10	72	55	76
	20	72	65	90
	40	72	72	100
VQA	1	72	5	7
	2.5	72	20	28
	5	72	30	42
	7.5	72	50	69
	10	72	61	85
	20	72	67	93
	40	72	72	100
Clinical Specimen 1	1	72	11	15
	2.5	72	20	28
	5	72	38	53
	10	72	49	68
	20	72	69	96
	40	72	69	96

Specimen	Nominal concentration (copies/mL)	No. Replicates	No. Positives	Positivity Rate (%)
Clinical Specimen 2	1	72	8	11
	2.5	72	17	24
	5	71	27	38
	10	72	47	65
	20	72	62	86
	40	72	72	100
Cell Culture Specimen	1	72	4	6
	2.5	72	17	24
	5	72	30	42
	10	72	46	64
	20	72	64	89
	40	72	70	97

In addition, dilutions of cell culture stocks or clinical specimens representing the HIV-1 group M subtypes A, C-D, F-H, J, K, CRF- A/B, CRF-A/E, CRF-A/G, group O, and group N in negative human EDTA plasma were analyzed with one Xpert HIV-1 VL test kit lot and 24 replicates per concentration level. The assignment of the nominal concentration of the cell culture stocks and clinical specimens was determined using the Abbott RealTi*m*e HIV-1 assay. Hit rate analysis shows a positivity of > 95% for all subtypes and groups at 40 copies/mL as shown in Table 4.

Table 4. Xpert HIV-1 VL Test LOD Hit Rate Analysis for HIV-1 non- B Subtype Specimens in EDTA Plasma

Group	Subtype	Lowest Concentration Level >95% Hit Rate (copies/mL)	Hit Rate (%)
Group M	**A**	20	96
Group M	**C**	40	100
Group M	**D**	20	100
Group M	**F**	40	100
Group M	**G**	40	96
Group M	**H**	20	96
Group M	**J**	20	100
Group M	**K**	40	96
Group M	**CRF A/B**	20	100
Group M	**CRF A/E**	20	96
Group M	**CRF A/G**	40	96
Group N	**N/A**	10	100
Group O[a]	**N/A**	20	100
Group O[a]	**N/A**	20	100

Group	Subtype	Lowest Concentration Level >95% Hit Rate (copies/mL)	Hit Rate (%)
Group O[a]	N/A	10	100

[a] Three different isolates

19.2 Limit of Quantitation

The limit of quantitation (LOQ) is defined as the lowest concentration of HIV-1 RNA that is quantified with acceptable precision and trueness, and determined using total analytical error (TAE). The TAE was calculated using estimates determined through analysis of data from the LOD study (WHO and VQA standards) and the Precision/Reproducibility study according to CLSI guideline E17-A2.[19]

The TAE for the dilutions that had an observed concentration at or near the assay limit of detection 40 copies/mL (1.60 $\log_{10}$) are presented in Table 5. TAE was estimated by two different methods. The results of the TAE analysis demonstrate that the Xpert HIV-1 VL test can determine 40 copies/mL(1.60 $\log_{10}$) with an acceptable trueness and precision i.e., the LOQ of the Xpert HIV-1 VL test is 40 copies/mL.

Table 5. HIV-1 VL Total Analytical Error (TAE) Estimates Log copies/mL

Specimen (Study)	DL Lot	N	Concentration (log copies/mL)		Bias	Total SD	TAE[a] Absolute Bias + (2xSD)	TAE[b] SQRT (2) x (2xSD)
			Expected	Observed				
Reference Material (Precision)	DL6	72	2.00	1.96	0.04	0.19	0.43	0.55
	DL7	71	2.00	1.91	0.09	0.19	0.46	0.53
	DL8	72	2.00	1.92	0.08	0.21	0.51	0.60
Reference Material (Precision)	DL6	70	1.60	1.56	0.04	0.22	0.48	0.62
	DL7	71	1.60	1.53	0.08	0.28	0.64	0.80
	DL8	71	1.60	1.54	0.06	0.22	0.50	0.62
WHO (LOD)	DL6	24	1.60	1.53	0.07	0.23	0.52	0.65
	DL7	24	1.60	1.39	0.21	0.24	0.68	0.67
	DL8	24	1.60	1.49	0.11	0.19	0.48	0.52
VQA (LOD)	DL6	24	1.60	1.61	0.00	0.18	0.37	0.51
	DL7	24	1.60	1.54	0.06	0.26	0.58	0.74
	DL8	24	1.60	1.58	0.02	0.26	0.54	0.73

[a] TAE calculated according to the Westgard model in CLSI EP17-A2 (Section 6.2).

[b] TAE based upon the difference between two measurements approach.

The results of the TAE analysis demonstrate that the Xpert HIV-1 VL test can determine 40 copies/mL (1.60 $\log_{10}$) with an acceptable trueness and precision.

19.3 Precision/Reproducibility

The precision/reproducibility of the Xpert HIV-1 VL test was determined by analysis of parallel dilutions of HIV-1 reference material (HIV-1 subtype B) in HIV-1 negative EDTA plasma. The reference material used was calibrated to the WHO HIV-1 3rd International Standard (NIBSC code: 10/152). The study was a two-site, blinded, comparative study using a seven-member panel of HIV-1 reference material in HIV-1 negative EDTA plasma with RNA concentrations that span the Xpert HIV-1 VL test quantitation range. Two operators at each of the two study sites tested one panel of twenty-one

samples once per day over six testing days. One site used an Infinity-80 instrument and the other site used GeneXpert Dx instruments. Three lots of Xpert HIV-1 VL test reagents were used for the study. Precision/Reproducibility was evaluated in accordance with "Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline" CLSI document EP5-A2.[21] The precision results for each kit lot and three kit lots combined are shown in Table 6.

Table 6. Xpert HIV-1 VL Test Precision per Lot and Total of Three Lots

Expected HIV-1 RNA Concentration (log_{10} copies/mL)	Total Precision per Lot						Total Precision 3 Lots	
	Lot 1		Lot 2		Lot 3		Total	
	SD[a]	CV[b]	SD[a]	CV[b]	SD[a]	CV[b]	SD[a]	CV[b]
1.60	0.24	58.6%	0.29	73.6%	0.23	57.6%	0.25	62.5%
2.00	0.20	48.8%	0.20	47.3%	0.22	53.1%	0.20	49.1%
3.00	0.10	22.6%	0.08	18.2%	0.10	22.6%	0.09	20.5%
4.00	0.06	13.7%	0.07	17.3%	0.09	19.8%	0.07	17.1%
5.00	0.06	13.8%	0.07	16.3%	0.08	17.7%	0.08	17.8%
6.00	0.05	12.4%	0.07	15.3%	0.07	16.2%	0.08	19.3%
7.00	0.06	14.3%	0.07	15.5%	0.09	21.5%	0.10	22.6%

[a] Total SD in log_{10}.

[b] "CV" is lognormal CV, as obtained using the formula: Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100CV(%) = percent coefficient of variation; SD = standard deviation; sqrt = square root

The reproducibility of the Xpert HIV-1 VL test was evaluated by using nested ANOVA with terms for Site/Instrument, Lot, Day, Operator/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 7).

Table 7. Xpert HIV-1 VL Test Contribution to Total Variance and Total Precision

HIV-1 RNA Concentration (log_{10} copies/mL)			Contribution to Total Variance SD (CV%)										Total Precision	
Expected	Actual (Average)	N[a]	Site		Lot		Day		Operator/ Run		Within-Run		Total	
			SD	(%)	SD	(%)	SD	(%)	SD	(%)	SD	(%)	SD	CV[b]
1.60	1.54	212	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.09	11.7%	0.23	88.3%	0.25	62.5%
2.00	1.93	215	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.04	4.8%	0.20	95.2%	0.20	49.1%
3.00	2.98	215	0.01	0.9%	0.01	1.2%	0.00	0.0%	0.01	2.6%	0.09	95.3%	0.09	20.5%
4.00	3.98	214	0.00	0.0%	0.01	3.5%	0.01	1.7%	0.02	9.1%	0.07	85.7%	0.07	17.1%
5.00	4.99	213	0.00	0.0%	0.04	21.8%	0.00	0.0%	0.03	15.0%	0.06	63.2%	0.08	17.8%
6.00	5.96	215	0.00	0.0%	0.05	42.1%	0.02	4.4%	0.02	6.9%	0.06	46.7%	0.08	19.3%
7.00	6.94	213	0.00	0.0%	0.07	45.3%	0.01	0.9%	0.02	5.3%	0.07	48.5%	0.10	22.6%

[a] Number of valid replicates within test range

[b] "CV" is lognormal CV, as obtained using the formula: Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100CV(%) = percent coefficient of variation; SD = standard deviation; sqrt = square root

19.4 Linear Range

The linear range of the Xpert HIV-1 VL test was determined by analysis of a nine member panel from 30 (1.48 log_{10}) to 1 x 10^7 (7 log_{10}) copies/mL prepared by parallel dilutions of HIV-1 reference material (HIV-1 subtype B) in HIV-1 negative EDTA plasma. The reference material used was calibrated to the WHO 3rd HIV-1 International Standard (NIBSC code: 10/152). Two operators tested the panel in replicates of three on three separate days using one kit lot. In addition, the same panel was tested in replicates of three on one day of testing using two additional kit lots resulting in a total 30 replicates per panel member. The linearity analysis was performed according to CLSI guideline EP06-A.[22] The combined results for all three lots are shown in Figure 8. The Xpert HIV-1 VL test is linear within a range 30 (1.5 log10) to 1 x 10E7 (7 log10) cp/mL with a R^2 value of 0.9935.

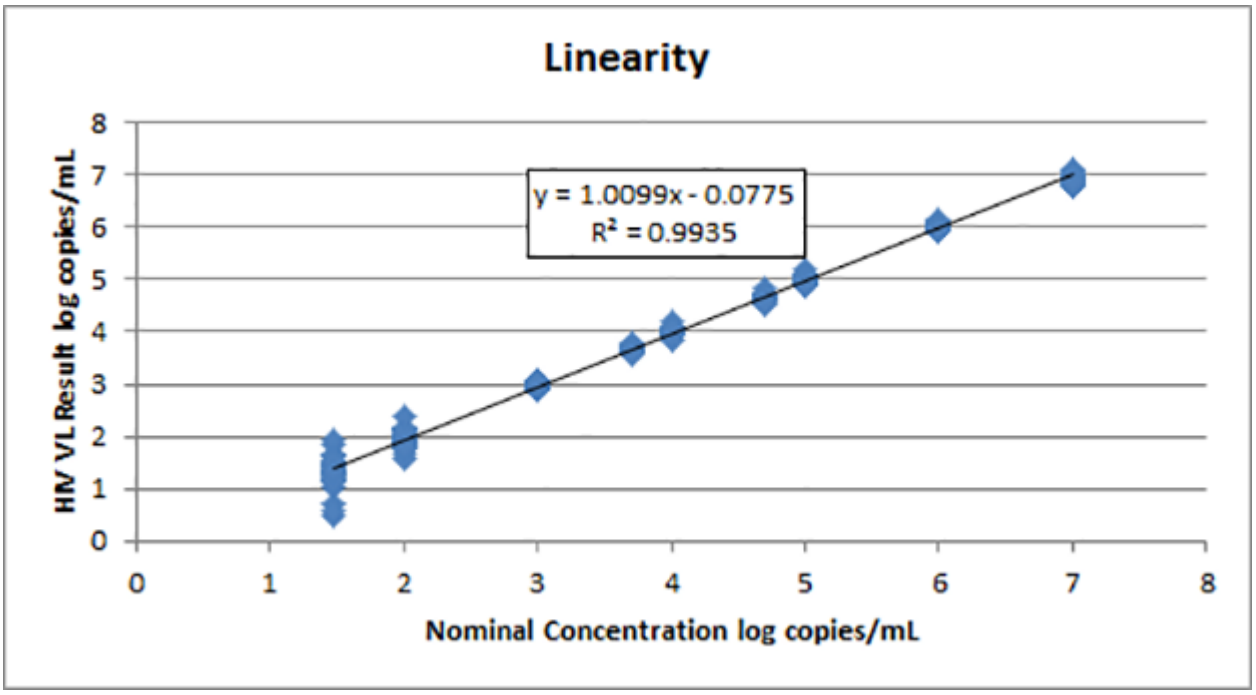


Figure 8. Linearity for the Xpert HIV-1 VL Test

19.5 Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert HIV-1 VL test was evaluated by testing cell culture supernatants representative of the HIV-1 Group M subtypes A-D, F-H, CRF A/G, and A/E; Group N; and Group O. The assignment of nominal concentrations to the cell culture supernatants was performed using the Abbott HIV-1 RealTi*m*e assay. Each cell culture supernatant was diluted to concentrations of 1 x 10^2, 1 x 10^4 and 1 x 10^6 copies/mL in HIV-1 negative EDTA plasma. Each concentration was tested in replicates of six on one day using one Xpert HIV-1 VL test kit lot. The mean log_{10} concentrations obtained with the Xpert HIV-1 VL test for all subtypes and groups were compared to nominal log_{10} concentrations. The results presented in Figure 9 show equivalent performance for all tested representatives of HIV-1 Group M subtypes and Group O. Mean log_{10} results for all tested subtypes and group O were within +/-0.5 log_{10} of the assigned input concentration.

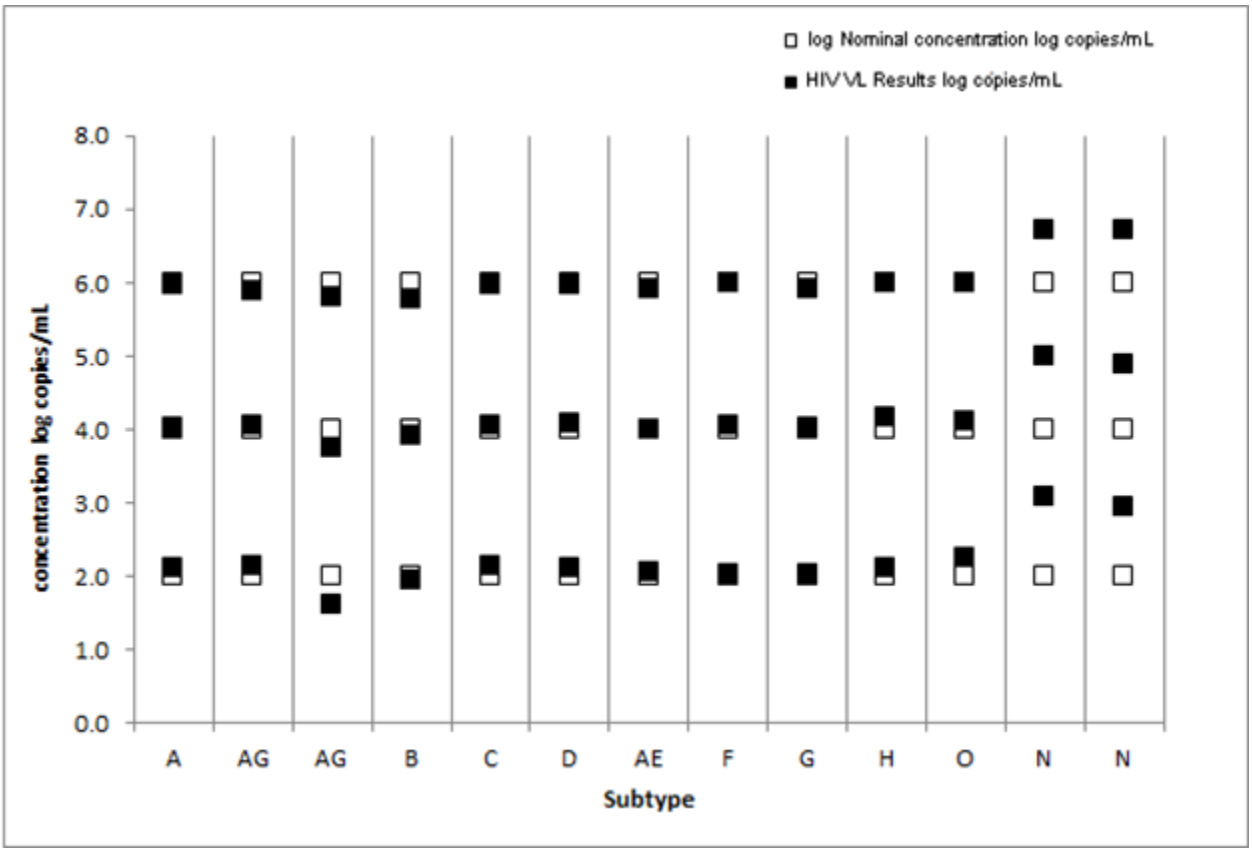


Figure 9. Inclusivity for the Xpert HIV-1 VL Test

19.6 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert HIV-1 VL test was evaluated by adding cultured organism at 5 x 10^4 particles or copies/mL input concentration into HIV-1 negative EDTA plasma and in plasma that contained 1000 copies/mL HIV-1 reference material (HIV-1 subtype B). Tested organisms are listed in Table 8.

Table 8. Analytical Specificity Organisms

Human Immunodeficiency virus 2
Human T-cell lymphotropic virus 1
Human T-cell lymphotropic virus 2
Candida albicans
Cytomegalovirus
Epstein-Barr virus
Hepatitis A virus
Hepatitis B virus
Hepatitis C virus
Herpes simplex virus 1
Herpes simplex virus 2
Human herpes virus 6
Influenza A

Staphylococcus aureus

None of the organisms tested showed cross reactivity and all HIV-1 positive replicates resulted in a titer within ± 0.5 log of the HIV-1 positive control when tested using the Xpert HIV-1 VL test.

19.7 Potentially Interfering Substances

The susceptibility of the Xpert HIV-1 VL test to interference by elevated levels of endogenous substances, by drugs prescribed to HIV-1 infected patients, and autoimmune disease markers was evaluated. HIV-1 negative EDTA plasma and plasma that contained 1000 copies/mL HIV-1 reference material (HIV-1 subtype B) were tested.

Elevated levels of the endogenous substances listed in Table 9 did not interfere with the quantification of the Xpert HIV-1 VL test or impact the test specificity.

Table 9. Endogenous Substances and Concentration Tested

Substance	Tested Concentration
Albumin	9 g/dL
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Human DNA	0.4 mg/dL
Triglycerides	3000 mg/dL

The drug components as presented in Table 10 did not interfere with the quantification of the Xpert HIV-1 VL test or impact the test specificity when tested at three times peak level concentrations in five drug pools.

Table 10. Drug Pools Tested

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

Testing of specimens from five individuals positive for an autoimmune disease marker—systemic lupus erythematosus (SLE), anti-nuclear antibody (ANA) or rheumatoid factor (RF)—showed no interference using the Xpert HIV-1 VL test.

19.8 Anti-coagulant Equivalence (EDTA, PPT-EDTA, and ACD)

For each anti-coagulant EDTA, PPT-EDTA, and ACD, specimens from 25 matched HIV-1 positive individuals and 25 matched HIV-1 negative specimens were collected and tested using one kit lot of the Xpert HIV-1 VL test.

As shown in Figure 10 and Figure 11, equivalent performance of the Xpert HIV-1 VL test was shown for EDTA versus ACD anti-coagulant and EDTA versus PPT-EDTA anti-coagulant. All HIV-1 positive specimens collected in ACD or PPT-EDTA media produced concentrations of HIV-1 RNA within ±0.5 $\log_{10}$ copies/mL of the HIV-1 positive specimen collected in EDTA media when tested using the Xpert HIV-1 VL test. All 25 matched HIV-1 negative specimens were not detected by the assay.

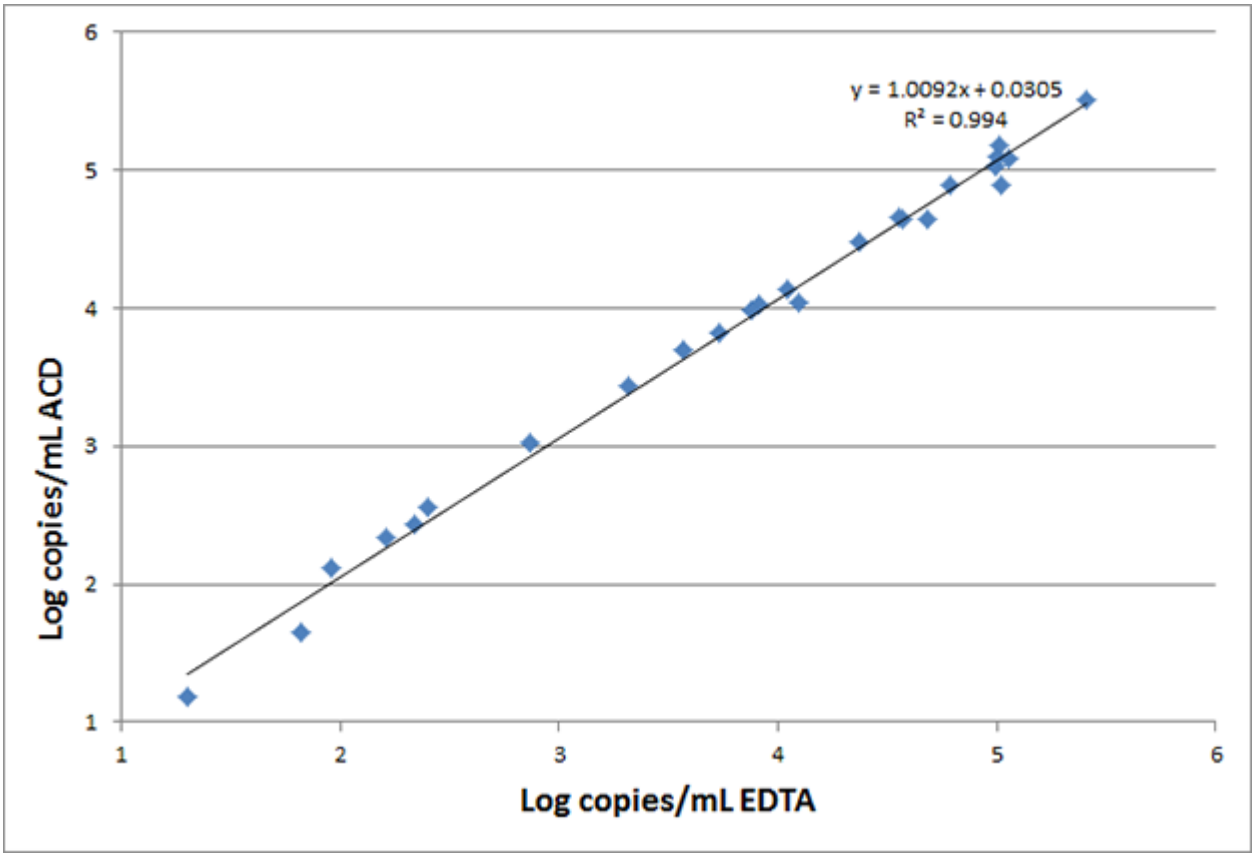


Figure 10. Scatterplot of Log copies/mL ACD versus Log copies/mL EDTA

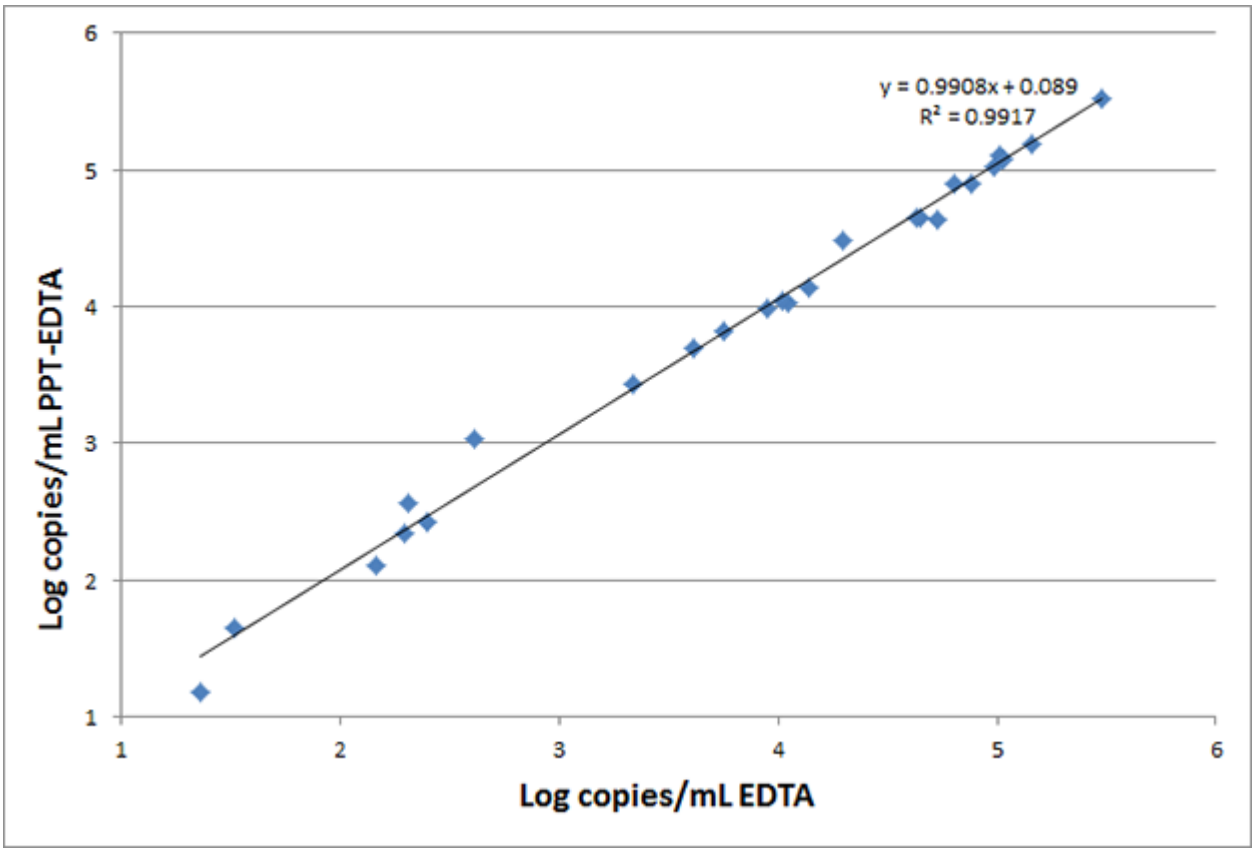


Figure 11. Scatterplot of Log copies/mL PPT-EDTA versus Log copies/mL EDTA

20 Limitations

- Good laboratory practices and changing gloves between handling specimens are recommended to avoid contamination of specimens or reagents.
- 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 EDTA and ACD plasma. Testing of other specimen types with this test may lead to inaccurate results.
- 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.

21 Performance Characteristics – Clinical Performance

21.1 Specificity

The specificity of the Xpert HIV-1 VL test was evaluated using 109 EDTA plasma specimens from HIV-1 negative blood donors. None of the 109 specimens tested were detected by the Xpert HIV-1 VL test equating to 100% specificity (95% CI = 96.7–100.0).

21.2 Method Correlation

A multi-site study was conducted to evaluate the performance of the Xpert HIV-1 VL test relative to the Abbott HIV-1 RealTi*m*e assay (Comparator) using fresh and frozen human plasma specimens collected from HIV-1 infected individuals. Of the 724 eligible specimens, each from unique individuals, 519 (71.8%) were collected from male subjects. The average age was 44.5 ± 11.3 years with an age range of 18 to 83 years.

Of the 724 specimens, 390 were within the quantitation range of both assays including 47 HIV-1 Group M non-B subtypes including A-like, C and C-like, D, F, G, H, J, AE, AG and various other circulating recombinant forms (CRFs). The Deming regression shows very good correlation between the Xpert HIV-1 VL test and the comparator method with a slope of 1.0589 and intercept of 0.1771. The R^2 was 0.9696.

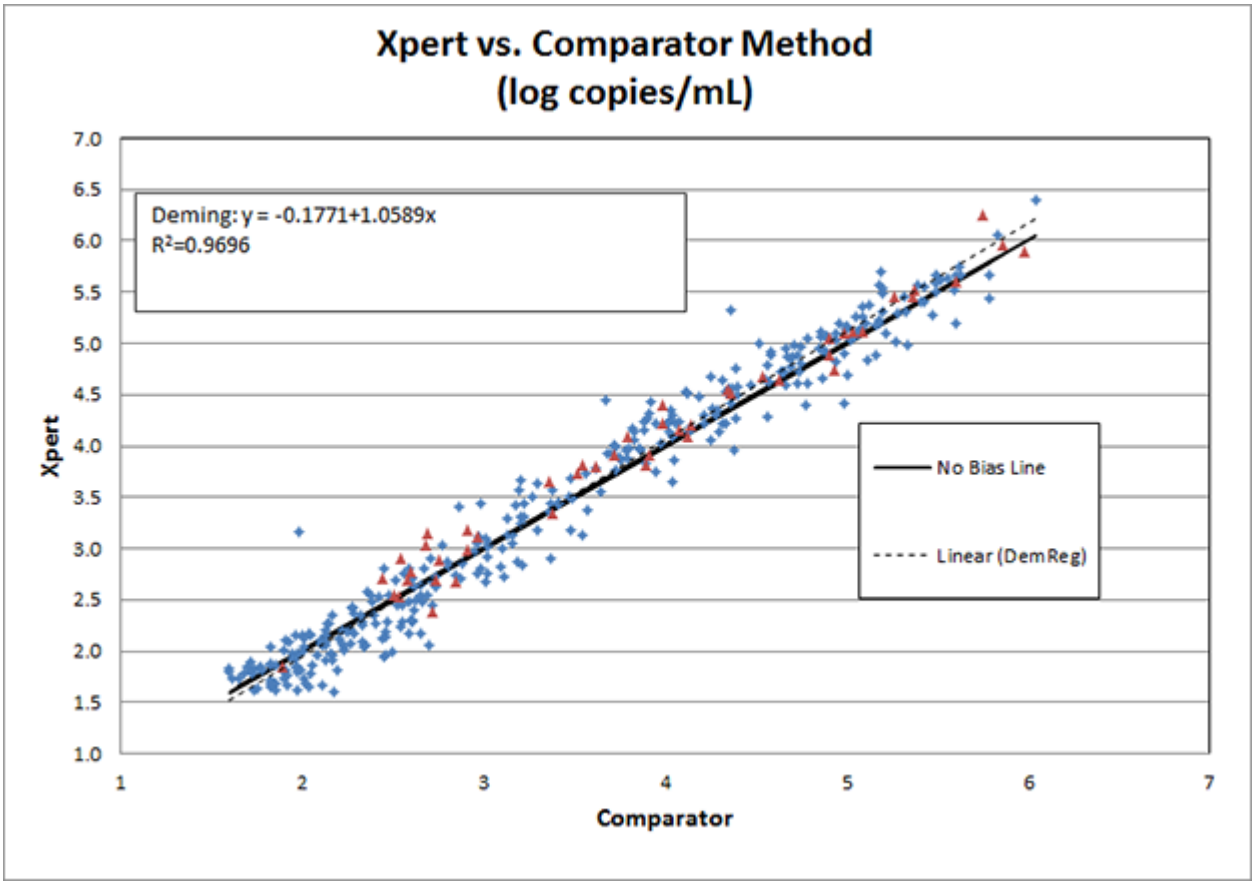


Figure 12. Performance of the Xpert HIV-1 VL Test Relative to a Comparator Method

22 References

1. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;220:868–871.
2. Popovic M, Sarngadharan MG, Read E, et al. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-I) from patients with AIDS and pre-AIDS. *Science* 1984;224:497–500.
3. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-I) from patients with AIDS and at risk for AIDS. *Science* 1984;224:500–503.
4. Curran JW, Jaffe HW, Hardy AM, et al. Epidemiology of HIV infection and AIDS in the United States. *Science* 1988;239:610–616.
5. Schochetman G, George JR, editors. *AIDS testing: a comprehensive guide to technical, medical, social, legal, and management issues*. 2nd ed. New York: NY Springer-Verlag; 1994.
6. Nduati R, John G, Mbori-Ngacha D, et al. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *Journal of the American Medical Association* 2000;283:1167–1174.
7. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics *in vivo*: virion clearance rate, infected cell life-span, and viral generation time. *Science* 1996; 271:1582–1586.
8. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, Lifson JD, Bonhoeffer S, Nowak MA, Hahn BH, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995; 373:117–122.
9. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995; 373:123–126.
10. Katzenstein DA, Hammer SM, Hughes MD, Gundacker H, Jackson JB, Fiscus S, Rasheed S, Elbeik T, Reichman R, Japour A, Merigan TC, Hirsch MS. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimeter. AIDS Clinical Trials Group Study 175 Virology Study Team. *N Engl J Med* 1996; 335:1091–1098.
11. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, Kingsley LA, Todd JA, Saah AJ, Detels R, Phair JP, Rinaldo CR, Jr. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997; 126:946–954.
12. 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.
13. O'Brien WA, Hartigan PM, Martin D, Esinhart J, Hill A, Benoit S, Rubin M, Simberkoff MS, Hamilton JD. Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. *N Engl J Med* 1996; 334:426–431.
14. Ruiz L, Romeu J, Clotet B, Balague M, Cabrera C, Sirera G, Ibanez A, Martinez-Picado J, Raventos A, Tural C, Segura A, Foz M. Quantitative HIV-1 RNA as a marker of clinical stability and survival in a cohort of 302 patients with a mean CD4 cell count of 300 x 10(6)/l. *Aids* 1996; 10:F39–44.
15. Saag MS, Holodniy M, Kuritzkes DR, O'Brien WA, Coombs R, Poscher ME, Jacobsen DM, Shaw GM, Richman DD, Volberding PA. HIV viral load markers in clinical practice. *Nat Med* 1996; 2:625–629.
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).
18. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
19. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).
20. Clinical and Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*; Approved Guideline. Document EP17-A2 (Second Edition). Wayne, PA: Clinical Laboratory Standards Institute; 2012
21. Clinical and Laboratory Standards Institute. *Evaluation of Precision Performance of Clinical Chemistry Devices*; Approved Guideline. Document EP5-A2.
22. Clinical and Laboratory Standards Institute. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*; Approved Guideline. NCCLS document EP06-A [ISBN 1-56238-498-8]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2003.

23 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

24 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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

Contact Information

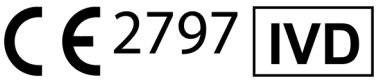
United States	France
Telephone: + 1 888 838 3222	Telephone: + 33 563 825 319
Email: techsupport@cepheid.com	Email: support@cepheideurope.com

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

25 Table of Symbols

Symbol	Meaning
	Catalog number
	CE marking – European Conformity
	For In Vitro Diagnostic Use Only
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Country of manufacture
	Control
	Expiration date
	Temperature limitation
	Biological risks

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden



26 Revision History

Description of Changes: From 301-3068 Rev. L to Rev. M

Purpose: To align with the requirements of Regulation (EU) 2017/746 and other applicable updates.

Section	Description of Change
Trademark,Patents and Copyright Statements	Updated per legal requirements.
Throughout	Changed instances of "assay" used as a brand name to "test". Changed instances of “HIV-1 VL” as product name to “Xpert HIV-1 VL”. Removed icons in left margin and updated formatting per technical publications standards.
5	Added *GeneXpert Edge System User’s Guide*.
8, 14	Added GeneXpert Edge System.
9	Added reference to instrument system cleaning and disinfecting instructions.
13, 15	Separated procedures for GeneXpert Dx System and GeneXpert Infinity System.
16	Added *GeneXpert Edge System User’s Guide* to note.
17	Added GeneXpert Edge System screen shots.
16.2	Updated Figure 2 per technical publications standard. Added *GeneXpert Edge System User’s Guide*.
26	Revision History added.