WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: Aptima HIV-1 Quant Dx Assay
WHO Reference number: PQDx 0236-078-00

Aptima HIV-1 Quant Dx Assay with product codes **PRD-03000 (PRD-03002, PRD-03001), 303014, PRD-03003 and PRD-03000B** , and instrument Panther system with product code **303095**, manufactured by **Hologic, Inc**, **CE regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 21 December 2017.

Summary of WHO prequalification assessment for Aptima HIV-1 Quant Dx Assay

	Date	Outcome
Prequalification listing	21-Dec-2017	listed
Dossier assessment	N/A	MR
Site inspection(s) of quality management system	1 to 2-Jul-2015	MR
Product performance evaluation	14-Dec-2017	MR

MR: Meets Requirements
NA: Not Applicable

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which 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.

Version	Summary of amendment	Date of report amendment
1.0-4.0	Editorial changes to the initial public report for listing.	21-Dec-2017
5.0	1. Transfer of assay reagents and equipment used to manufacture Aptima HIV-1 Quant Dx Assay from the Willow Court facility to the Genetic Center Drive facility.	07-Feb-2018

	2. Labelling change to include additional print on the existing labelling stating the new manufacturing site.	
6.0	Adding Dried Blood Spots (DBS) sample type to the Aptima HIV-1 Quant Dx Assay. Labelling change to include additional print on the finished label of the Aptima DBS Extraction Buffer which states the Manchester manufacturing address.	27-Oct-2020
7.0	Addition of the new part number, PRD-03000B. PRD-03000 provides 100 tests accompanied by one box of calibrators and controls. PRD-03000B consists of 500 tests and two boxes of calibrators and controls to minimize wastage.	20-Jan-2021
8.0	1. Addition of a secondary manufacturing site (Manchester, UK). 2. Labelling change to include an additional stamp on the existing labelling stating the new manufacturing site. 3. Addition of an automated fill process.	24-Jun-2021
9.0	Closure of commitment to amend the IFU to include a warning that only EDTA plasma were validated specimen types.	16-Sep-2021

Intended use:

According to the claim of intended use from Hologic, Inc., for the Aptima HIV-1 Quant Dx assay package insert "*the Aptima HIV-1 Quant Dx and DBS Supplement to the Aptima HIV-1 Quant Dx Assay is an in vitro nucleic acid amplification test for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) RNA groups M, N, and O on the fully automated Panther system. It is intended for use as an aid in the diagnosis of HIV-1 infection, as a confirmation of HIV-1 infection, and as an aid in clinical management of patients infected with HIV-1.*

The Aptima HIV-1 Quant Dx assay may be used as an aid in the diagnosis of HIV-1 infection, including acute or primary infection. Presence of HIV-1 RNA in the plasma or serum of patients without antibodies to HIV-1 is indicative of acute or primary HIV-1 infection.

The Aptima HIV-1 Quant Dx assay may be used as a supplemental test for specimens that have repeat reactive results with approved HIV immunoassays. If the specimen is reactive in the Aptima HIV-1 Quant Dx assay, HIV-1 infection is confirmed.

The Aptima HIV-1 Quant Dx assay may also be used in conjunction with clinical presentation and other laboratory markers for disease prognosis in HIV-1 infected individuals. The Aptima HIV-1 Quant Dx assay may be used as an aid in monitoring the effect of antiretroviral treatment by measuring changes in the concentration of HIV-1 RNA in plasma.

*When the Aptima HIV-1 Quant Dx assay is used as an aid in the diagnosis of HIV-1 infection, performance for qualitative results is established with both plasma and serum specimens. * When used as an aid in monitoring the effect of antiretroviral therapy, performance for*

quantitative results is established with plasma specimens only. Serum specimens may not be used for quantitative results.

This assay is not intended for use in screening blood or plasma donors."

According to the claim of intended use from Hologic, Inc., DBS Supplement to the Aptima HIV-1 Quant Dx Assay, "*the Aptima HIV-1 Quant Dx assay is an in vitro nucleic acid amplification test for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) RNA groups M, N, and O on the fully automated Panther system. It is intended for use as an aid in the diagnosis of HIV-1 infection, as a confirmation of HIV-1 infection, and as an aid in clinical management of patients infected with HIV-1.*

In addition, the Aptima HIV-1 Quant Dx assay may be used as an aid in the diagnosis of acute or primary HIV-1 infection. Presence of HIV-1 RNA in the plasma, serum, or blood of patients without antibodies to HIV-1 is indicative of acute or primary HIV-1 infection. The Aptima HIV-1 Quant Dx assay may be used as a supplemental test for specimens that have repeat reactive results with approved HIV immunoassays. If the specimen is reactive in the Aptima HIV-1 Quant Dx assay, HIV-1 infection is confirmed.

The Aptima HIV-1 Quant Dx assay may also be used in conjunction with clinical presentation and other laboratory markers for disease prognosis in HIV-1 infected individuals. The Aptima HIV-1 Quant Dx assay may also be used as an aid in EID of HIV-1 infection in infants below 18 months of age using DBS. The Aptima HIV-1 Quant Dx assay may be used as an aid in monitoring the effect of antiretroviral treatment by measuring changes in the concentration of HIV-1 RNA in plasma and DBS samples.

When the Aptima HIV-1 Quant Dx assay is used as an aid in the diagnosis of HIV-1 infection, performance for qualitative results is established with both plasma and serum specimens as well as DBS samples from infants below 18 months of age. When used as an aid in monitoring the effect of antiretroviral therapy, performance for quantitative results is established with plasma and DBS specimens only. Serum specimens may not be used for quantitative results.

This assay is not intended for use in screening blood or plasma donors."

Assay description:

According to the claim of intended use from Hologic, Inc, "*Aptima HIV-1 Quant Dx assay involves three main steps, which all take place in a single tube on the Panther system: target capture, target amplification by transcription-mediated amplification (TMA), and detection of the amplification products (amplicon) by the fluorescent labeled probes (torches).*

During target capture, viral nucleic acids are isolated from specimens. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Capture oligonucleotides hybridize to highly conserved regions of the HIV-1 genome, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

Target amplification occurs via TMA, which is a transcription-mediated nucleic acid amplification method that utilizes two enzymes, MMLV (Moloney murine leukemia virus) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Aptima HIV-1 Quant Dx assay utilizes the TMA method to amplify two regions of HIV-1 RNA (pol and LTR). Amplification of these specific regions is achieved using specific primers which are designed to amplify HIV-1 groups M, N, and O. The primer design and the dual target approach ensure accurate detection and quantitation of HIV-1.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to the amplicon in real-time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and it will emit a signal at a specific wavelength when excited by a light source. As more torches hybridize to amplicon a higher fluorescent signal is generated. The time taken for the fluorescent signal to reach a specified threshold is proportional to the starting HIV-1 concentration. Each reaction has an internal calibrator/internal control (IC) that controls for variations in specimen processing, amplification, and detection. The concentration of a sample is determined by the Panther system software using the HIV-1 and IC signals for each reaction and comparing them to calibration information."

Test kit contents:

Aptima HIV-1 Quant Dx Assay Box (Product code PRD-03000, 100 tests) and Aptima HIV-1 Quant Dx Assay Box (Product code PRD-03000B, 500 tests)

Component	Description	Quantity (for 100 tests)	Quantity (for 500 tests)
A qHIV-1 Amplification Reagent	Non-infectious nucleic acids dried in buffered solution.	1 vial (lyophilized)	5 x 1 vial (lyophilized)
E qHIV-1 Enzyme Reagent	Reverse transcriptase and RNA polymerase dried in HEPES buffered	1 vial (lyophilized)	5 x 1 vial (lyophilized)
PRO qHIV-1 Promoter Reagent	Non-infectious nucleic acids dried in buffered solution.	1 vial (lyophilized)	5 x 1 vial (lyophilized)
AR qHIV-1 Amplification Reconstitution Solution	Aqueous solution containing glycerol and preservatives	1 vial x 7.2 ml	5 vials x 7.2 ml
ER qHIV-1 Enzyme Reconstitution Solution	HEPES buffered solution containing a surfactant and glycerol	1 vial x 5.8 ml	5 vials x 5.8 ml
PR qHIV-1 Promoter Reconstitution Solution	Aqueous solution containing glycerol and preservatives.	1 vial x 4.5 ml	5 vials x 4.5 ml
TCR qHIV-1 Target Capture Reagent	Nucleic acids in a buffered salt solution containing solid phase, noninfectious nucleic acids, and Internal Calibrator.	1 vial x 72.0 ml	5 vials x 72.0 ml
Reconstitution Collars	N/A	3	15
Master Lot Barcode Sheet	N/A	1 sheet	5

Note: PRD-03000 contains 1 assay box, 1 calibrator kit, and 1 controls kit. Calibrator and Control kits are available separately if desired.

Aptima HIV-1 Quant Dx Controls Kit (Product code PRD-03002)

Component	Description	Quantity (100 tests configuration)	Quantity (500 tests configuration)
NC qHIV-1 Negative Control	HIV-1 negative defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.	5 vials x 1.5 ml	10 vials x 1.5 ml
LPC qHIV-1 Low Positive Control	Non-infectious HIV-1 Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.	5 vials x 1.5 ml	10 vials x 1.5 ml
HPC qHIV-1 High Positive Control	Non-infectious HIV-1 Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.	5 vials x 1.5 ml	10 vials x 1.5 ml
Control Barcode Label		-	-

Aptima HIV-1 Quant Dx Calibrator Kit (Product code PRD-03001)

Component	Description	Quantity (for 100 tests)	Quantity (for 500 tests)
PCAL qHIV-1 Positive Calibrator	Transcript in buffered solution.	5 vials x 2.5 ml	10 vials x 2.5 ml
Calibrator Barcode Label		-	-

Aptima DBS Extraction Buffer (Product code: PRD-04772).

Component	Description	Quantity (for 100 tests)
DBS extraction buffer	Phosphate buffered solution containing detergent	1 vial x 104 ml

Items required but not provided, available separately.
Note: Materials available from Hologic have product codes listed, unless otherwise specified.

Component	Product code	Description
Instrument		
Panther system	303095	Instrument
Consumables		
Panther Run Kit for Real Time Assays	PRD-03455 (5000 tests)	Assay Fluids kit Multi-tube Units Panther waste bag kit Panther waste bin cover
Aptima Assay Fluids kit (Universal Fluids kit)	303014 (1000 tests)	Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent
Aptima Specimen Diluent	PRD 03003	Optional
Multi-tube units (MTUs)	104772-02	
Panther Waste Bag Kit	902731	
Panther Waste Bin Cover	504405	
Tips, 1000 µL conductive, liquid sensing, Tecan	10612513 (Tecan)	
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	-	
Disposable, powderless gloves	-	
Replacement non-penetrable caps	103036A	
Reagent replacement caps:	CL0041	

• Amplification, Enzyme, and Promoter reagent reconstitution bottles (100 caps) • TCR bottle (100 caps)	CL0040	
Component	**Product code**	**Description**
Plastic-backed laboratory bench covers	-	
Lint-free wipes	-	
Pipettor	-	
Tips	-	
Primary collection tubes (ACD, EDTA, PPT, SST, Serum) of the following dimensions may be used: • 13 mm x 100 mm • 13 mm x 75 mm • 16 mm x 100 mm	-	
DBS Extraction materials.		
Aptima specimen aliquot tubes	503762	Pack of 100
Transport tube caps	504415	Pack of 100
Calibrated Pipettors	-	-
Aerosol barrier pipette tips	-	-
Equipment		
Centrifuge	-	
Vortex mixer	-	

Storage:

Aptima HIV-1 Quant Dx Assay Box should be stored at 2-8 °C.
Aptima HIV-1 Quant Dx Calibrator Kit should be stored at -15°C to -35°C.
Aptima HIV-1 Quant Dx Controls Kit should be stored at -15°C to -35°C.
Aptima DBS Extraction Buffer should be stored at 15°C to 40°C.

Shelf-life upon manufacture:

24 months.

Warnings/Limitations:

Refer to the attached instructions of use (IFU).

Prioritization for prequalification

Based on the established eligibility criteria, Aptima HIV-1 Quant Dx Assay was given priority for WHO prequalification assessment.

Product dossier assessment

In accordance with the WHO procedure for abbreviated prequalification assessment, Hologic Inc. was not required to submit a product dossier for Aptima HIV-1 Quant Dx Assay 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.

Commitment for prequalification

As a commitment for prequalification, the manufacturer committed to amend the IFU to include a warning that only EDTA plasma has been validated by 30 April 2021. The submitted evidence was acceptable, and the issue was closed.

Manufacturing site inspection

In accordance with the WHO procedure for abbreviated prequalification assessment, a shortened inspection with fewer inspectors was conducted at the site(s) of manufacture (10210 Genetic Center Drive, San Diego, 92121, USA, 6333 Sequence Drive, San Diego, 92121 USA, and 10808 Willow Court, San Diego, 92127, USA) of Aptima HIV-1 Quant Dx Assay in July 2015 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 6 August 2015.

Commitment for prequalification:

Include WHO in the reporting system to ensure information is communicated to WHO on any adverse event or safety corrective action.

Product performance evaluation

Aptima HIV-1 Quant Dx Assay was evaluated from 28 October 2015 to 6 April 2016 and from 29 November 2016 to 23 July 2017. From this evaluation, we drew the following conclusions.

Aptima HIV-1 Quant Dx Assay is a laboratory-based in vitro nucleic acid amplification test for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) RNA groups M, N, and O on the fully automated Panther system. It is intended for use as an aid in the diagnosis of HIV-1 infection, as a confirmation of HIV-1 infection, and as an aid in clinical management of patients infected with HIV-1.

This type of assay requires additional laboratory equipment for specimen preparation and reagent storage and cannot be performed in laboratories with limited facilities. The instrument requires a stable source of electricity and has a one meter square footprint. Furthermore, substantial training was considered essential to obtaining accurate results.

Analytical specimens:
The assay's precision of measurement was verified. In this evaluation the precision of measurement was found to be acceptable, all %CV were found to be < 6%.

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 < 0.2 from an ideal value of 1. R^2 values were all >0.97 indicating good correlation between the reference method and the assay under evaluation

The limit of detection was verified. In this evaluation the LOD was estimated to be 31.57 IU/ml (95% Fiducial limits: 20.63-75.37); 11.05 copies/ml (95% Fiducial limits: 7.22-26.38, conversion factor is 0.35, 1 IU = 0.35 copies).

No carry-over was detected.

Clinical specimens – first evaluation:

In this performance evaluation on a panel of 432 specimens, we found a bias -0.065 log10 copies/ml (limits of agreement: -1.158; 1.027) compared to the reference results.

Correlation was found to be within range (R^2 =0.9476, P<0.1).

Sensitivity for treatment failure at 1000 copies/ml 93.91% (95% CI: 90.00-96.34)
Specificity for treatment failure at 1000 copies/ml was 100.00% (95% CI: 98.13-100.00)

In this study, the invalid rate was 3.3%.

Performance characteristics	
Analytical performance	
Limit of Detection	31.57 IU/ml (95% Fiducial limits: 20.63-75.37); 11.05 copies/ml (95% Fiducial limits: 7.22-26.38)
Linearity	Verified in subtypes: A, B, C, D, and AG Linearity for all subtypes was determined to be acceptable. All slopes were within were < 0.2 from an ideal value of 1. R^2 values were all > 0.97
Carry-over	0%
Clinical performance	
Bias	-0.065 log10 copies/ml (limits of agreement: -1.158; 1.027)
Correlation	Within range (R2 =0.9476, P<0.1)
Sensitivity for virological failure at 1000 copies/ml	93.91% (95% CI: 90.00-96.34)
Specificity for virological failure at 1000 copies/ml	100.00% (95% CI: 98.13-100.00)
Invalid rate	3.29%

Key operational characteristics	
Validated specimen types	For quantitative measurements: Tubes containing EDTA or Acid Citrate Dextrose (ACD) anticoagulants or Plasma Preparation Tubes (PPTs). For qualitative determination: Tubes containing EDTA or ACD anticoagulants, or PPTs, or Serum tubes, or Serum separator tubes (SSTs).
Number of steps	10
Time to result	3h:30 minutes (preparation and loading: 50 minutes; test: 2h:40 minutes for the first result; 5 results every 5 minutes thereafter).
In-use stability of reagents	30 days for reagents 20 hours for single use vials

Labelling

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

1. Labels

1.1 Outside Box label

REF PRD-03000

Aptima® HIV-1 Quant Dx Assay

For the detection and quantitation of HIV-1 RNA

Aptima® HIV-1 Quant Dx Assay

A	Amplification Reagent (Nucleic Acids)	1 EA
E	Enzyme Reagent (Enzyme)	1 EA
PRO	Promoter Reagent (Nucleic Acids)	1 EA
AR	Amplification Reconstitution Solution (Aqueous Solution)	1 EA
ER	Enzyme Reconstitution Solution (HEPES Buffered Solution)	1 EA
PROR	Promoter Reconstitution Solution (Aqueous Solution)	1 EA
TCR	Target Capture Reagent (Nucleic Acid in a Buffered Solution)	1 EA
	Reconstitution Collars	1 Package

Hologic, Inc., 10210 Genetic Center Drive, San Diego, CA 92121 USA

LBL-07604 Rev. 001

Aptima® HIV-1 Quant Dx Assay

For the detection and quantitation of HIV-1 RNA

Σ 100 IVD 1434 2°C 8°C

HOLOGIC®

For U.S. Export only

EC REP
Hologic BVBA, Da Vincilaan 5,
1930 Zaventem, Belgium

(01) 15420045509092
(17) 180718
(10) 123456AH

123456AH

2018-07-18

Manufactured at Hologic, Ltd. Oaks Business Park, Crewe Road, Wythenshawe, Manchester, M23 9HZ, UK

Part Number and Revision: AW-13495 Rev.003

1.2 Master lot bar code

HOLOGIC® Aptima® HIV-1 Quant Dx Assay

MASTER LOT BARCODE SHEET

AW-11231 Rev. 003

Σ 100 IVD

Master Lot No.: LOT 123456 PV

Master Lot Exp Date: 2021-10-26

Master Lot Date of Manufacture: 2019-02-25

28123456261021200

A

Amplification Reagent

LOT 111111 2021-10-26 2019-02-24

2801111111261021

E

Enzyme Reagent

LOT 222222 2021-10-26 2019-02-23

2802222222261021

PRO

Promoter Reagent

LOT 333333 2021-10-26 2019-02-22

2803333333261021

TCR

Target Capture Reagent

LOT 444444 2021-10-26 2019-02-21

2805444444261021

AR

Amplification Reconstitution Solution

LOT 555555 2021-10-26 2019-02-20

2816555555261021

ER

Enzyme Reconstitution Solution

LOT 666666 2021-10-26 2019-02-19

2817666666261021

PROR

Promoter Reconstitution Solution

LOT 777777 2021-10-26 2019-02-18

2818777777261021

Calibrator Coefficient 1

280000001122334455

Calibrator Coefficient 2

280000012233445566

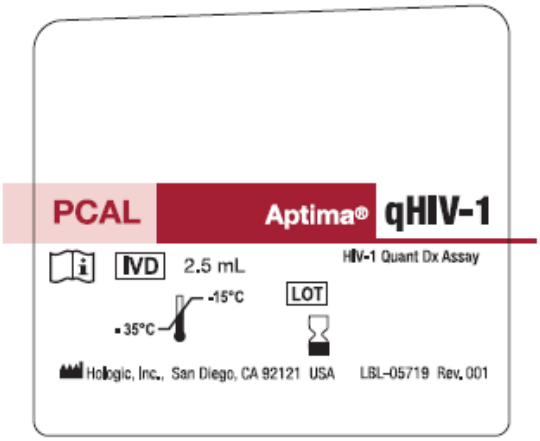
Calibrator Coefficient 3

280000103344556677

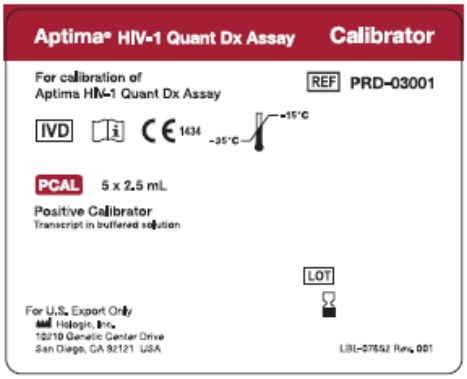
Calibrator Coefficient 4

280000114455667788

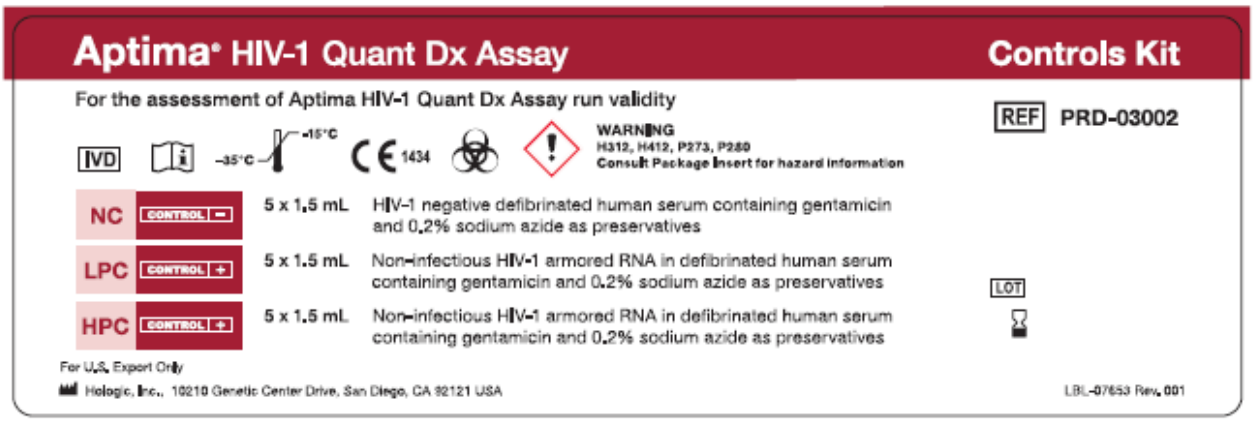
1.3 Calibrator label



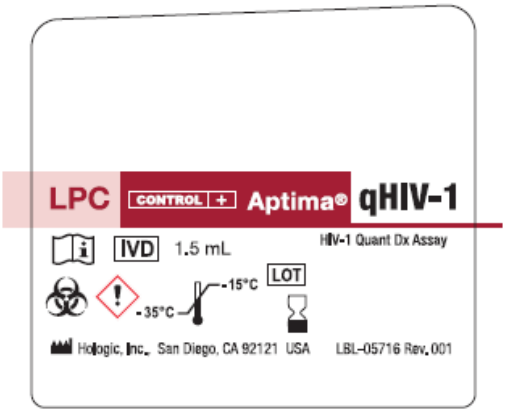
1.4 Calibrator tray label



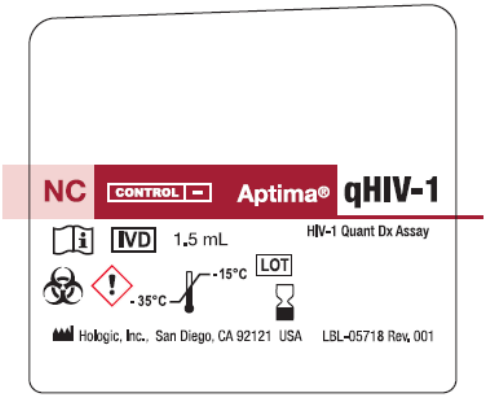
1.5 Control kit Tray label



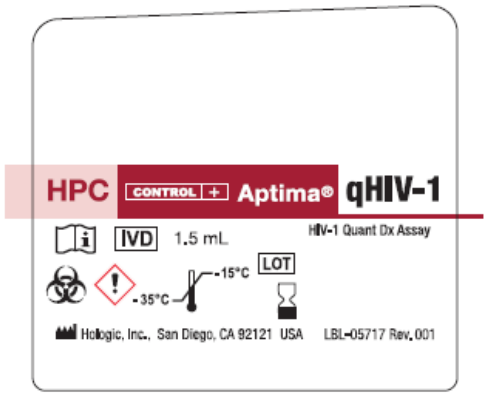
1.6 Low positive control label



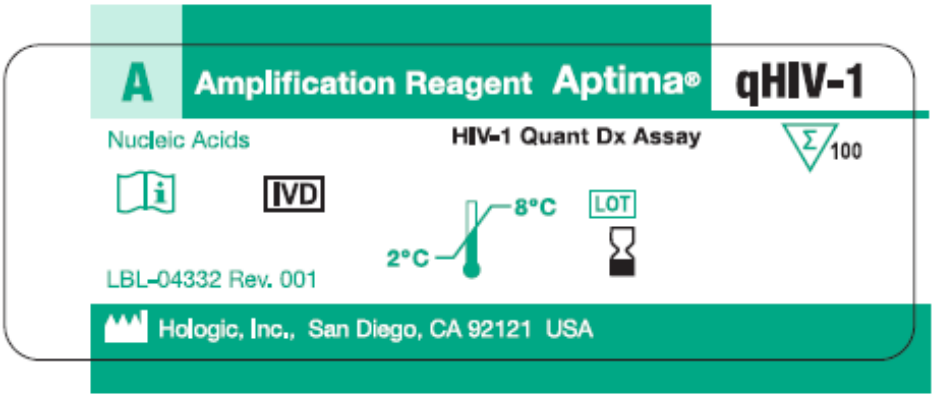
1.7 Negative control label



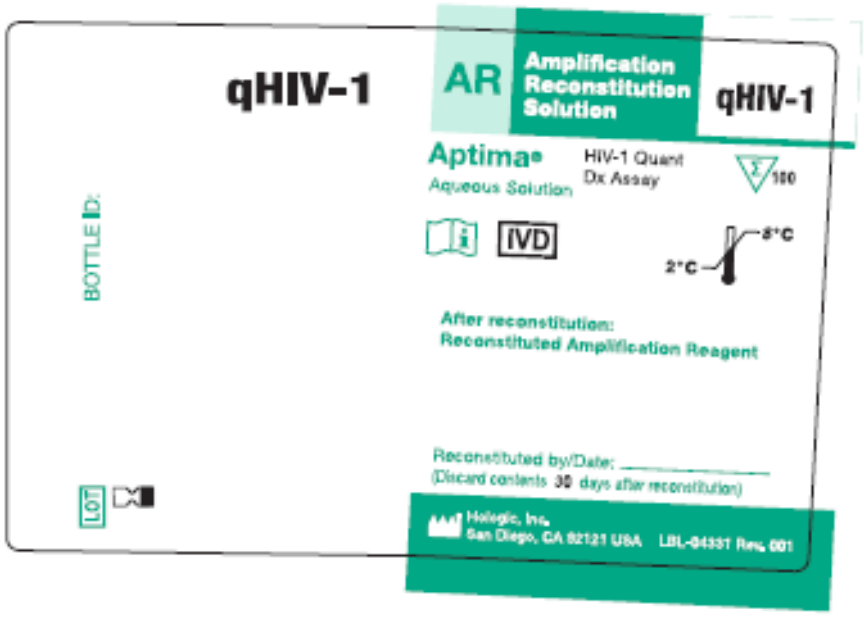
1.8 High positive control



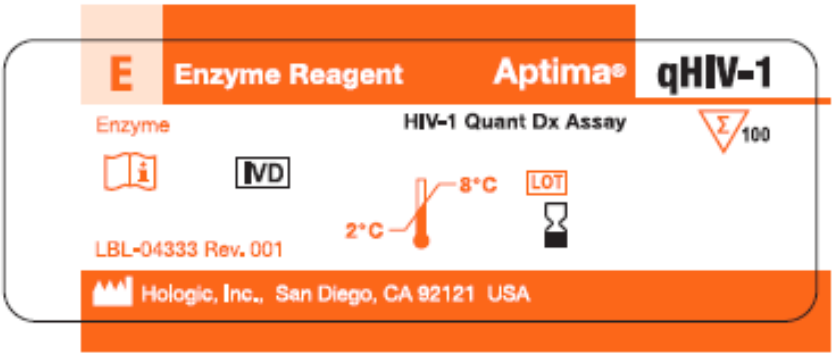
1.9 Amplification reagent



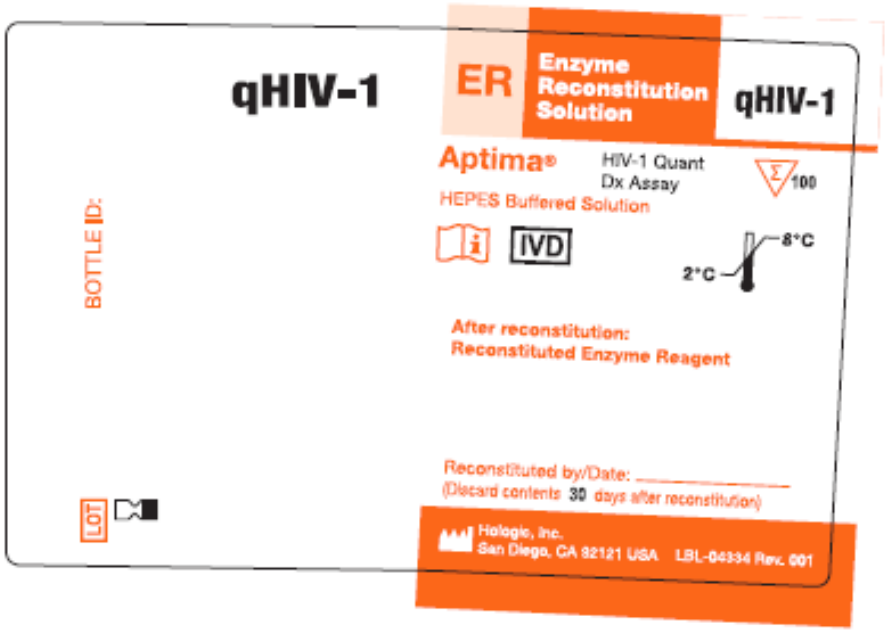
1.10 Amplification reconstitution solution



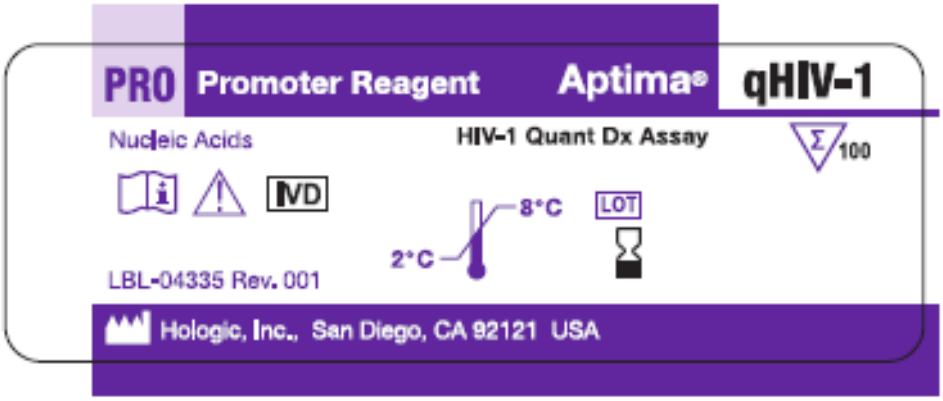
1.11 Enzyme reagent label



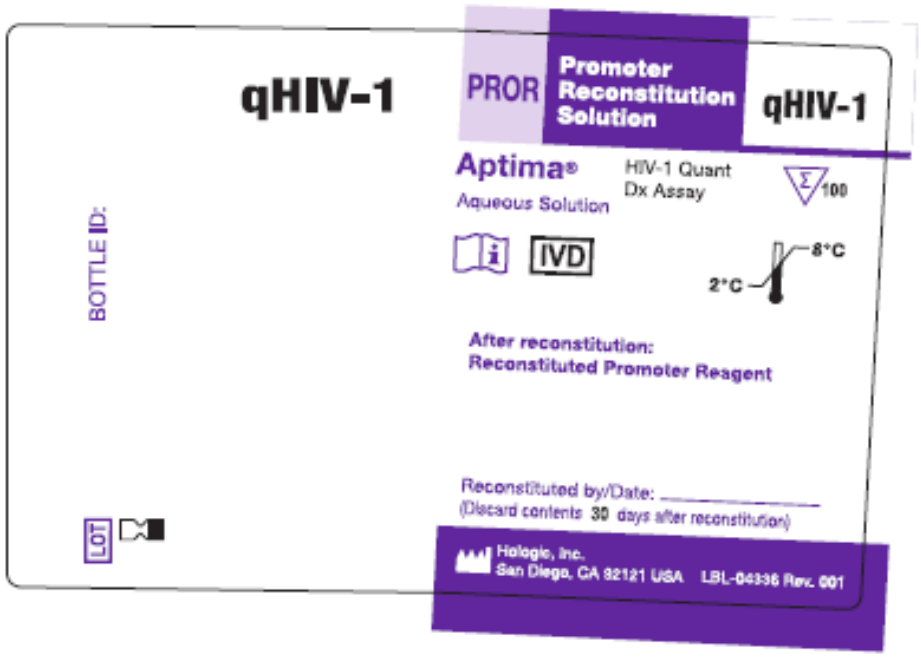
1.12 Enzyme reconstitution solution label



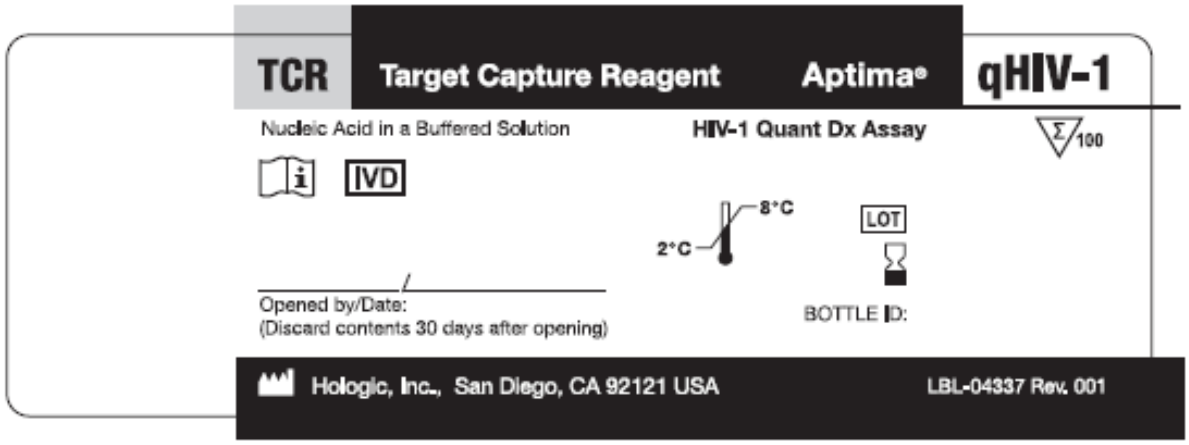
1.13 Promotor reagent label



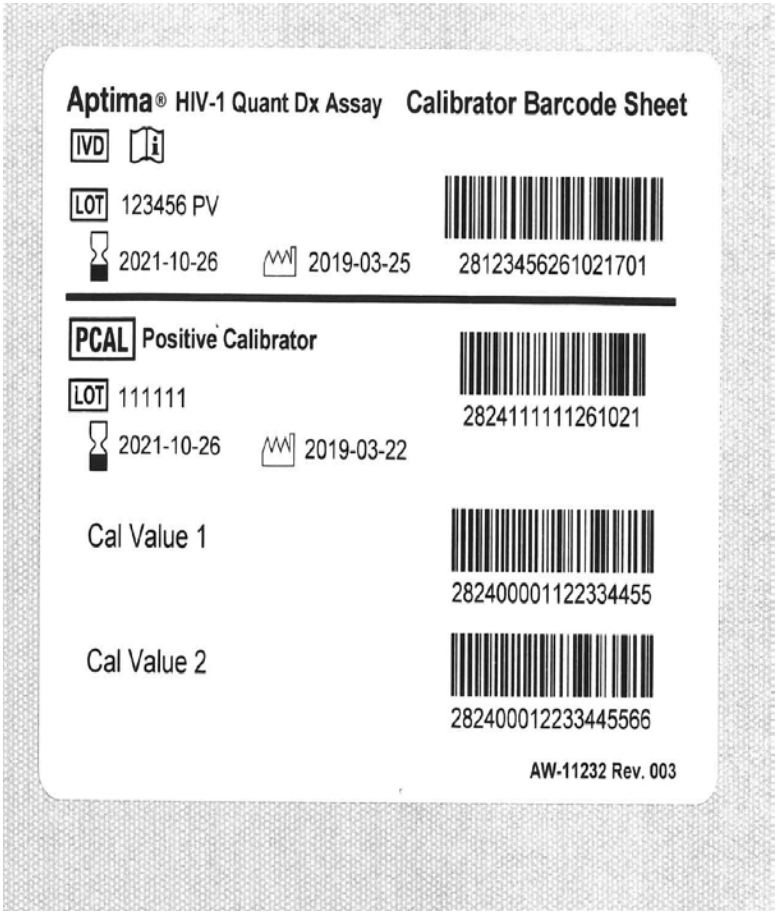
1.14 Promotor reconstitution solution label



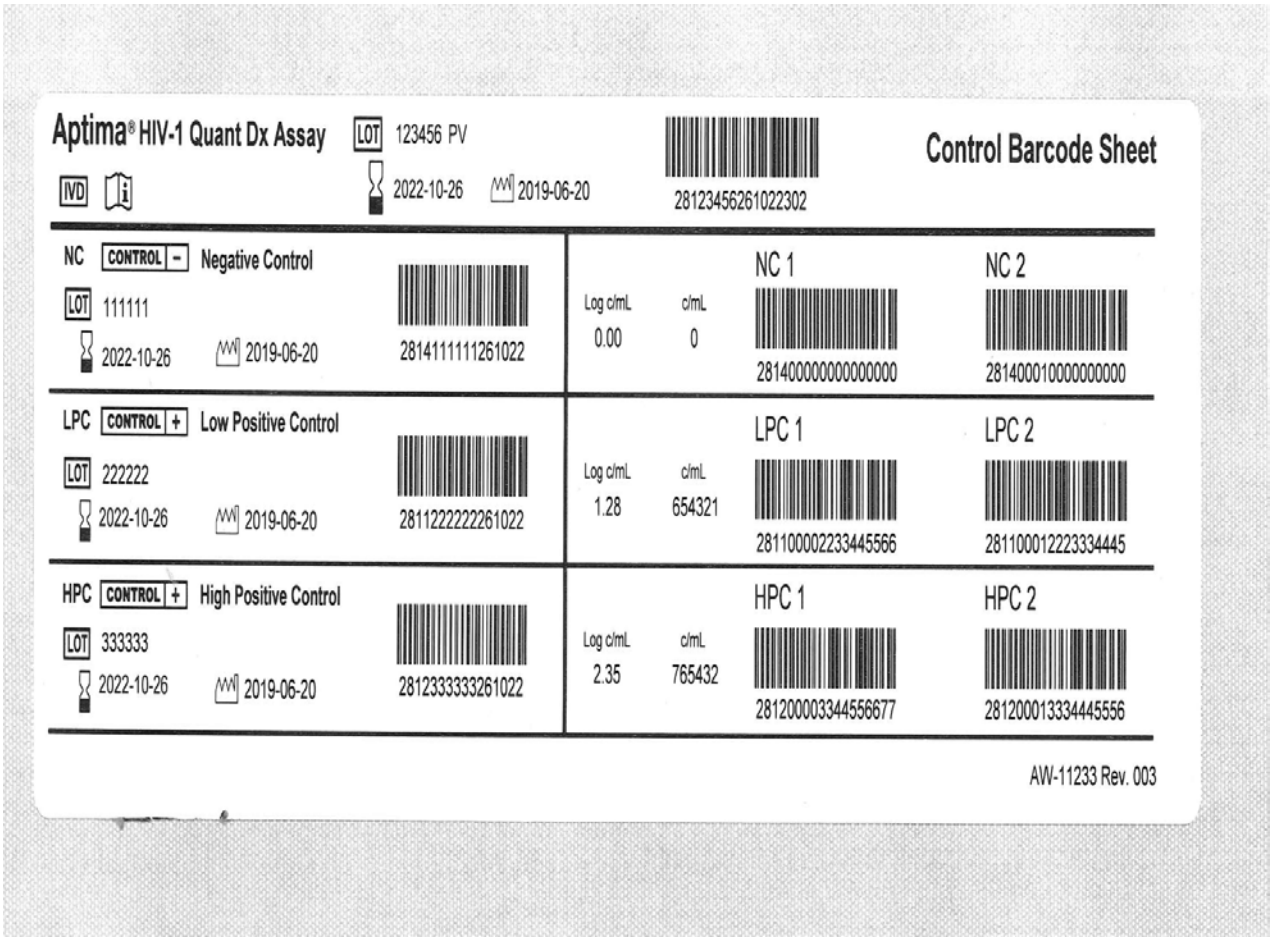
1.15 Target Capture reagent label



1.16 Calibrator bar code sheet



1.17 Controls bar code sheet



1.18 DBS extraction buffer packaging box

REF PRD-04772

Aptima®
DBS Extraction Buffer

Aptima®
DBS Extraction Buffer

DEB 1 x 104 mL
Phosphate Buffered Solution
containing detergent

Aptima®
DBS Extraction Buffer
For the extraction of dried blood spots

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

IVD

15°C 40°C

For U.S. Export only

HOLOGIC®

2.0 Instructions for use[1]

[1] 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.

2.1 Aptima HIV-1 Quant Dx Assay

Aptima™ HIV-1 Quant Dx Assay

For *in vitro* diagnostic use.

For US export only.

General Information

Intended Use

The Aptima HIV-1 Quant Dx assay is an *in vitro* nucleic acid amplification test for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) RNA groups M, N, and O on the fully automated Panther™ system. It is intended for use as an aid in the diagnosis of HIV-1 infection, as a confirmation of HIV-1 infection, and as an aid in clinical management of patients infected with HIV-1.

The Aptima HIV-1 Quant Dx assay may be used as an aid in the diagnosis of HIV-1 infection, including acute or primary infection. Presence of HIV-1 RNA in the plasma or serum of patients without antibodies to HIV-1 is indicative of acute or primary HIV-1 infection. The Aptima HIV-1 Quant Dx assay may be used as a supplemental test for specimens that have repeat reactive results with approved HIV immunoassays. If the specimen is reactive in the Aptima HIV-1 Quant Dx assay, HIV-1 infection is confirmed.

The Aptima HIV-1 Quant Dx assay may also be used in conjunction with clinical presentation and other laboratory markers for disease prognosis in HIV-1 infected individuals. The Aptima HIV-1 Quant Dx assay may be used as an aid in monitoring the effect of antiretroviral treatment by measuring changes in the concentration of HIV-1 RNA in plasma.

When the Aptima HIV-1 Quant Dx assay is used as an aid in the diagnosis of HIV-1 infection, performance for qualitative results is established with both plasma and serum specimens.* When used as an aid in monitoring the effect of antiretroviral therapy, performance for quantitative results is established with plasma specimens only. Serum specimens may not be used for quantitative results.

This assay is not intended for use in screening blood or plasma donors.

Summary and Explanation of the Test

Epidemiological studies identified human immunodeficiency virus type 1 (HIV-1) as the etiological agent of acquired immunodeficiency syndrome (AIDS) (1-7). HIV can be transmitted by sexual contact, exposure to infected blood or blood products, or through mother-to-child transmission (8). Within 3 to 6 weeks of exposure to HIV, infected individuals generally develop a brief, acute syndrome characterized by flu-like symptoms and associated with high levels of viremia in the peripheral blood (9-12). In most infected individuals, this early phase is followed by an HIV-specific immune response and a decline of plasma viremia, usually within 4 to 6 weeks of the onset of symptoms (13-14). After seroconversion, infected individuals typically enter a clinically stable, asymptomatic phase that can last for years (15-17). The asymptomatic period is characterized by persistent, low-level plasma viremia (18) and a gradual depletion of CD4+ T lymphocytes. This depletion leads to severe immunodeficiency, multiple opportunistic infections, malignancies, and death (19). Although levels of virus in the peripheral blood are relatively low during the asymptomatic phase of the infection, virus replication and clearance appear to be dynamic processes in which high rates of virus production and infection of CD4+ cells are balanced by equally high rates of virus clearance, death of infected cells, and replenishment of CD4+ cells, resulting in relatively stable levels of both plasma viremia and CD4+ cells (20-22).

Quantitative measurements of HIV in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV-associated disease, and shown that reductions in plasma virus levels may be associated with decreased risk of clinical progression (23-25). Virus levels in the peripheral blood can be quantitated by

*Note: Dried blood spot (DBS) specimens may also be used with the Aptima HIV-1 Quant Dx assay. For full description of intended use and information for DBS specimens, refer to the Dried Blood Spot Supplement to the Aptima HIV-1 Quant Dx Assay package insert (AW-17780).

measurement of the HIV p24 antigen in serum, by quantitative culture of HIV from plasma, or by direct measurement of viral RNA in plasma using nucleic acid amplification or signal amplification technologies (26-30).

Current detection of HIV-1 infection is primarily based on serologic testing for antibodies and/or p24 antigen by an immunoassay. The US Centers for Disease Control recommends the use of an antibody and RNA test to diagnose acute HIV infections (31). Although sensitivity of HIV-1 antibody and p24 antigen detection has improved, there still exists a window period between the time of infection and the time of detection by serological markers. This window period is dependent on the sensitivity of the serological test used. One estimate (32) suggests that 4th generation p24 antigen/antibody assays may detect infection when the HIV-1 RNA concentration reaches 14,000 copies/mL. The limit of detection of the Aptima HIV-1 Quant Dx assay is significantly lower than 14,000 copies/mL and may detect the presence of HIV-1 earlier than HIV immunoassays.

Molecular techniques such as transcription mediated amplification (TMA) have been widely used to amplify nucleic acids (31). TMA uses specific target capture and isothermal amplification to detect nucleic acids in multiple infectious pathogens (32).

The Aptima HIV-1 Quant Dx assay, through TMA, utilizes multiple, long primers that target several regions of the HIV-1 genome in order to compensate for the high mutation rate and multiple potential mutations at the target region.

Principles of the Procedure

The Aptima HIV-1 Quant Dx assay involves three main steps, which all take place in a single tube on the Panther system: target capture, target amplification by transcription-mediated amplification (TMA), and detection of the amplification products (amplicon) by the fluorescent labeled probes (torches).

During target capture, viral nucleic acids are isolated from specimens. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Capture oligonucleotides hybridize to highly conserved regions of the HIV-1 genome, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

Target amplification occurs via TMA, which is a transcription-mediated nucleic acid amplification method that utilizes two enzymes, MMLV (Moloney murine leukemia virus) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Aptima HIV-1 Quant Dx assay utilizes the TMA method to amplify two regions of HIV-1 RNA (pol and LTR). Amplification of these specific regions is achieved using specific primers which are designed to amplify HIV-1 groups M, N, and O. The primer design and the dual target approach ensure accurate detection and quantitation of HIV-1.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to the amplicon in real-time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and it will emit a signal at a specific wavelength when excited by a light source. As more torches hybridize to amplicon a higher fluorescent signal is generated. The time taken for the

fluorescent signal to reach a specified threshold is proportional to the starting HIV-1 concentration. Each reaction has an internal calibrator/internal control (IC) that controls for variations in specimen processing, amplification, and detection. The concentration of a sample is determined by the Panther system software using the HIV-1 and IC signals for each reaction and comparing them to calibration information.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther System Operator's Manual* prior to performing this assay.

Laboratory Related

C. CAUTION: The controls for this assay contain human plasma. The plasma is negative for hepatitis B surface antigen (HBsAg), antibodies to HCV, antibodies to HIV-1 and HIV-2, and HIV antigen when tested with US Food and Drug Administration licensed procedures. In addition, the plasma is nonreactive for HCV RNA and HIV-1 RNA when tested with licensed nucleic acid tests using pooled samples. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions (35-37).

D. Only personnel adequately trained in the use of the Aptima HIV-1 Quant Dx assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

E. Use only supplied or specified disposable laboratory ware.

F. Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

G. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

H. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations (35-38). Thoroughly clean and disinfect all work surfaces.

I. The controls contain sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing sodium azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

J. Good standard practices for molecular laboratories include environmental monitoring. To monitor a laboratory's environment, the following procedure is suggested.

1. Obtain a cotton-tipped swab and pair with the Aptima Specimen Aliquot Tube (SAT).
2. Label each SAT appropriately.
3. Fill each SAT with 1 mL of Aptima Specimen Diluent.

4. To collect the surface samples, lightly moisten a swab with nuclease free deionized water.
5. Swab the surface of interest using a top to bottom vertical motion. Rotate the swab approximately one-half turn while swabbing the location.
6. Immediately place the swab sample into the tube and gently swirl the swab in the diluent to extract potential swabbed materials. Press the swab on the side of the transport tube to extract as much liquid as possible. Discard the swab and cap the tube.
7. Repeat steps for remaining swab samples.
8. Test swab with molecular assay.

Specimen Related

K. Specimens may be infectious. Use Universal Precautions (35-37) when performing this assay. Proper handling and disposal methods should be established according to local regulations (38). Only personnel adequately trained in the use of the Aptima HIV-1 Quant Dx assay and trained in handling infectious materials should perform this procedure.

L. Only plasma with anticoagulants EDTA and ACD have been evaluated.

M. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

N. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

Assay Related

O. Quantitative results of the Aptima HIV-1 Quant Dx assay have been evaluated with EDTA and ACD plasma. **Serum may not be used to obtain quantitative results.** Qualitative results have been evaluated with both plasma and serum.

P. Do not use the reagent kit, the calibrator, or the controls after the expiration date.

Q. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Assay fluids can be from different lot numbers. Controls and the calibrator can be from different lot numbers.

R. Avoid microbial and nuclease contamination of reagents.

S. Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Reagent Storage and Handling Requirements* and *Panther System Test Procedure* for more information.

T. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

U. Some reagents in this kit are labeled with risk and safety symbols.

Note: *Hazard Communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

HIV VL Kit Controls
Sodium Azide 0.2%
Human Serum 95-100%

WARNING
H312 - Harmful in contact with skin
H412 - Harmful to aquatic life with long lasting effects
P273 - Avoid release to the environment
P280 - Wear protective gloves/protective clothing/eye protection/face protection

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for reagents, controls, and calibrator.

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
qHIV-1 Amplification Reagent	2°C to 8°C		
qHIV-1 Amplification Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 Enzyme Reagent	2°C to 8°C		
qHIV-1 Enzyme Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 Promoter Reagent	2°C to 8°C		
qHIV-1 Promoter Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 Target Capture Reagent	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 NC CONTROL – (Negative Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 LPC CONTROL + (Low Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 HPC CONTROL + (High Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 PCAL (Positive Calibrator)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours

[a] When reagents are removed from the Panther system, they should be immediately returned to their appropriate storage temperatures.

B. Discard any unused reconstituted reagents and target capture reagent (TCR) after 30 days or after the Master Lot expiration date, whichever comes first.

C. Reagents stored onboard the Panther system have 72 hours of onboard stability. Reagents can be loaded onto the Panther system up to 5 times. The Panther system logs each time the reagents are loaded.

D. After thawing the calibrator, the solution must be clear, i.e., not cloudy or have precipitates.

⚠ E. The Promoter Reagent and reconstituted Promoter Reagent are photosensitive. Protect these reagents from light during storage and preparation for use.

Specimen Collection and Storage

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

Note: *Only plastic secondary tubes are recommended for storage.*

Whole blood specimens collected in the following glass or plastic tubes may be used:

For quantitative measurements:

- Tubes containing EDTA or Acid Citrate Dextrose (ACD) anticoagulants or
- Plasma Preparation Tubes (PPTs).

For qualitative determination:

- Tubes containing EDTA or ACD anticoagulants, or
- PPTs, or
- Serum tubes, or
- Serum separator tubes (SSTs).

For serum, allow the clot to form before further processing.

A. Specimen Collection

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Separate the plasma or serum from the pelleted red blood cells following the manufacturer's instructions for the tube used. Plasma or serum can be tested on the Panther system in a primary tube or transferred to a secondarytube such as the Aptima Specimen Aliquot Tube. To obtain the 500 µl reaction volume, the minimum volume of plasma or serum for primary collection tubes is up to 1200 µL and for secondary tubes, the minimum volume is 700 µL. The following table identifies dead volume requirements for each primary and secondary tube type.

Tube (Size and Type)	Dead Volume on Panther
Aptima Sample Aliquot Tube (SAT)	0.2 mL
12x75 mm	0.5 mL
13x100 mm	0.5 mL
13x100 mm with Gel	0.3 mL
16x100 mm with Gel	0.7 mL

If not tested immediately, plasma and serum can be stored in accordance with the specifications below. If transferred to a secondary tube, plasma may be frozen at -20°C or -70°C, and serum may be frozen at -20°C. Do not exceed three freeze–thaw cycles to avoid affecting the result. Do not freeze specimens in EDTA, ACD, or serum primary collection tubes.

B. Specimen Storage Conditions

1. EDTA and ACD Plasma Specimens

For up to 24 hours after specimen collection, primary tubes containing centrifuged plasma may be stored at 2°C to 30°C (Figure 1, upper box). After 24 hours, plasma may be stored for a longer period of time under one of the following conditions (Figure 1, lower boxes):

- In the primary collection tube at 2°C to 8°C for up to 3 days,
- In the secondary tube at 2°C to 8°C for up to 5 days, or
- In the secondary tube at -20°C or -70°C for up to 90 days.

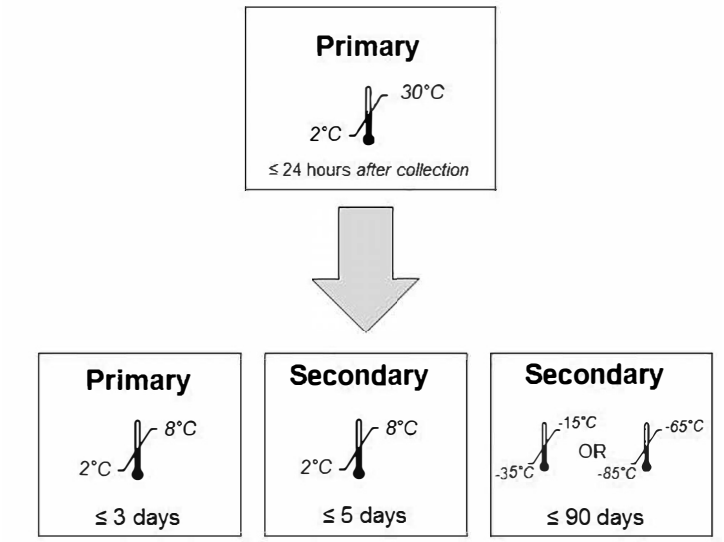


Figure 1. Storage Conditions for EDTA/ACD Tubes

2. PPT Specimens

   For up to 24 hours after specimen collection, PPTs containing centrifuged plasma may be stored at 2°C to 30°C (Figure 2, upper box). After 24 hours, plasma may be stored for a longer period of time under one of the following conditions (Figure 2, lower boxes):

   - In the PPT at 2°C to 8°C for up to 3 days,
   - In the secondary tube at 2°C to 8°C for up to 5 days, or
   - In the PPT or secondary tube at -20°C or -70°C for up to 90 days

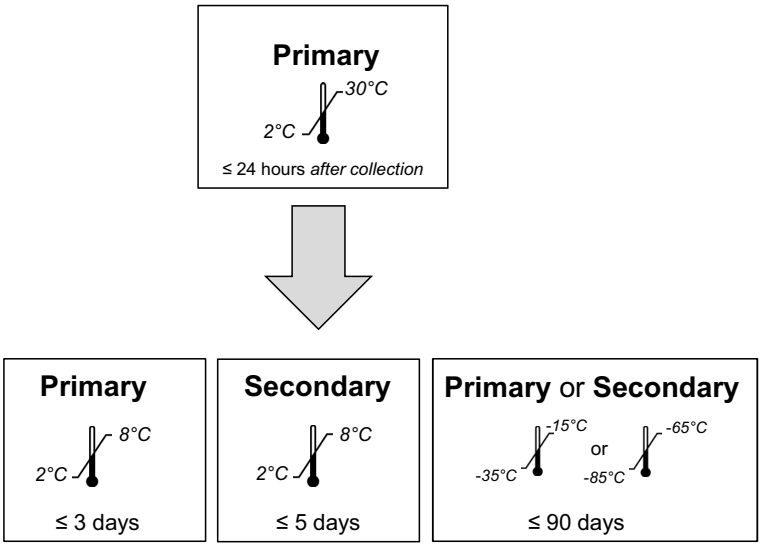


Figure 2. Storage Conditions for PPTs

3. Serum Tube Specimens

   For up to 24 hours after specimen collection, serum tubes containing centrifuged serum may be stored at 2°C to 30°C (Figure 3, upper box). After 24 hours, serum may be stored for a longer period of time under one of the following conditions (Figure 3, lower boxes):

   - In the serum tube at 2°C to 8°C for up to 5 days,
   - In the secondary tube at 2°C to 8°C for up to 5 days, or
   - In the secondary tube at -20°C for up to 7 days.

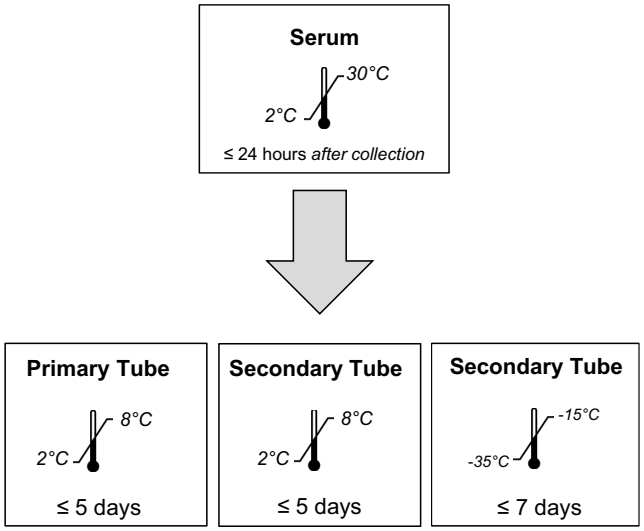


Figure 3. Storage Conditions for Serum Tubes

4. SST Specimens

   For up to 24 hours after specimen collection, SSTs containing centrifuged serum may be stored at 2°C to 30°C (Figure 4, upper box). After 24 hours, serum may be stored for a longer period of time under one of the following conditions (Figure 4, lower boxes):

   - In the SST at 2°C to 8°C for up to 5 days,
   - In the secondary tube at 2°C to 8°C for up to 5 days, or
   - In the secondary tube or SST at -20°C for up to 7 days.

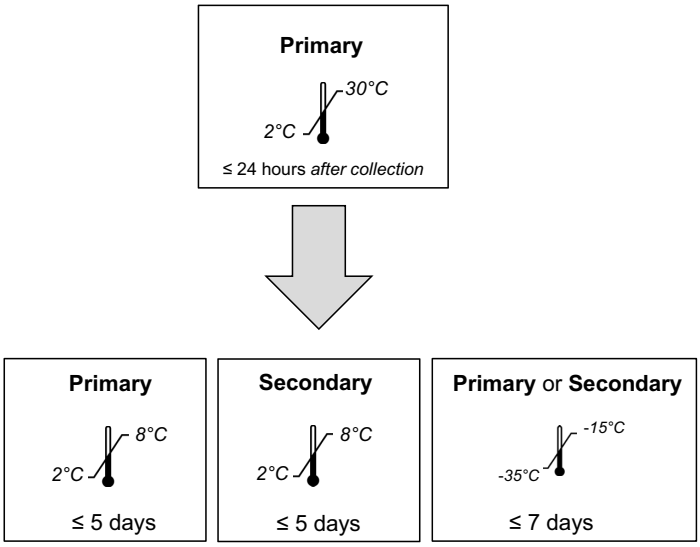


Figure 4. Storage Conditions SSTs

C. Dilution of Plasma Specimens

A plasma specimen may be diluted in the SAT or secondary tube for testing on the Panther system. See *Panther System Test Procedure*, step E.6 below for more information.

Note: *If a specimen is diluted, it should be tested immediately after dilution. Do not freeze a diluted specimen.*

⚠ *Dilution of plasma specimens may only be used for quantitative results. Do not dilute plasma samples for diagnostic results.*

Samples Onboard the Panther System

Samples may be left on the Panther system uncapped for up to a total of 8 hours. Samples may be removed from the Panther system and tested as long as the total time onboard does not exceed 8 hours prior to the pipetting of the sample by the Panther system.

Specimen Transport

Maintain sample storage conditions as described in *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima HIV-1 Quant Dx assay are listed below for the Panther system. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Aptima HIV-1 Quant Dx Assay Kit, 100 tests, Cat. No. PRD-03000 (1 assay box, 1 calibrator kit, and 1 controls kit)
Additional calibrators and controls may be ordered separately. See respective catalog numbers below.

Aptima HIV-1 Quant Dx Assay Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**qHIV-1 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**qHIV-1 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**qHIV-1 Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
AR	**qHIV-1 Amplification Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 7.2 mL
ER	**qHIV-1 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 5.8 mL
PROR	**qHIV-1 Promoter Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 4.5 mL
TCR	**qHIV-1 Target Capture Reagent** *Nucleic acids in a buffered salt solution containing solid phase, non-infectious nucleic acids, and Internal Calibrator.*	1 x 72.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Aptima HIV-1 Quant Dx Calibrator Kit (Cat. No. PRD-03001)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
PCAL	**qHIV-1 Positive Calibrator** *Transcript in buffered solution.*	5 x 2.5 mL
	Calibrator Barcode Label	—

Aptima HIV-1 Quant Dx Controls Kit (Cat. No. PRD-03002)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
NC	**qHIV-1 Negative Control** *HIV-1 negative defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 1.5 mL
LPC	**qHIV-1 Low Positive Control** *Non-infectious HIV-1 Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 1.5 mL
HPC	**qHIV-1 High Positive Control** *Non-infectious HIV-1 Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 1.5 mL
	Control Barcode Label	—

Materials Required But Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	—
Panther Run Kit for Real Time Assays (for real time assays only)	PRD-03455 (5000 tests)
Aptima Assay Fluids Kit (also known as Universal Fluids kit) *contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or, Panther System Run Kit *(when running non-real time-TMA assays in parallel with real time-TMA assays)* *contains MTUs, waste bags, waste bin covers, auto detect, and assay fluids*	303096 (5000 tests)
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—
Replacement non-penetrable caps	103036A
Reagent replacement caps	
Amplification, Enzyme, and Promoter reagent reconstitution bottles	*CL0041 (100 caps)*
TCR bottle	*CL0040 (100 caps)*
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tube (ACD, EDTA, PPT, SST, Serum) options:	
13 mm x 100 mm	—
13 mm x 75 mm	—
16 mm x 100 mm	—
Centrifuge	—
Vortex mixer	—

Optional Materials

Material	Cat. No.
Secondary tube options:	
12 mm x 75 mm	—
13 mm x 100 mm	—
16 mm x 100 mm	—
Aptima Specimen Aliquot Tubes (SATs) (100 pack)	503762
Transport Tube Cap (100 pack) *cap for SAT*	504415
Aptima Specimen Diluent	PRD-03003
Aptima Specimen Diluent Kit *contains specimen diluent, 100 SATs and 100 caps*	PRD-03478
Transfer pipets	—
Commercially available panels, for example: *HIV-1 from Quality Control for Molecular Diagnostics (QCMD) or College of American Pathologists (CAP) HIV viral load survey panel or SeraCare ACCURUN HIV Panels*	—
Cotton-tipped swabs	—
Tube rocker	—

Panther System Test Procedure

Note: *See the Panther System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (step A.1).
3. Clean any pipettors. Use the procedure described above (step A.1).

B. Calibrator and Controls Preparation

Allow the calibrator and controls to reach 15°C to 30°C prior to processing as follows:

1. Remove the calibrator and controls from storage (-15°C to -35°C) and place at 15°C to 30°C. Throughout the thawing process, gently invert each tube to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Option. Calibrator and control tubes may be placed on a tube rocker to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Note: *Avoid creating excessive foam when inverting the calibrator and controls. Foam compromises the level-sensing by the Panther system.*
2. When the tube contents have thawed, dry the outside of the tube with a clean, dry disposable wipe.
3. To prevent contamination, do not open the tubes at this time.

C. Reagent Reconstitution/Preparation of a New Kit

Note: *Reconstitution of reagents should be performed prior to beginning any work on the Panther system.*

1. To prepare Target Capture Reagent (TCR), perform the following:
   a. Remove the TCR from storage (2°C to 8°C). Check the lot number on the TCR bottle to make sure that it matches the lot number on the Master Lot Barcode Sheet.
   b. Immediately shake the TCR bottle vigorously 10 times. Allow the TCR bottle to remain at 15°C to 30°C to warm for at least 45 minutes. During this period, swirl and invert the TCR bottle at least every 10 minutes.

      Option. The TCR bottle may be prepared on a tube rocker by following these instructions: Remove the TCR from storage (2°C to 8°C) and immediately shake vigorously 10 times. Place the TCR bottle on a tube rocker and leave the TCR at 15°C to 30°C to warm for at least 45 minutes.
   c. Ensure all precipitate is in solution and the magnetic particles are suspended before use.
2. To reconstitute Amplification, Enzyme, and Promoter Reagents, perform the following:
   a. Remove the lyophilized reagents and corresponding reconstitution solutions from storage (2°C to 8°C). Pair each reconstitution solution with its lyophilized reagent.
   b. Ensure that the reconstitution solution and lyophilized reagent have matching label colors. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
      i. Open the lyophilized reagent vial by removing the metallic seal and rubber stopper.
      ii. Firmly insert the notched end of the reconstitution collar (black) onto the vial (Figure 5, Step 1).
      iii. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
      iv. Place the reconstitution solution bottle on a stable surface (i.e., bench). Then, invert the lyophilized reagent vial over the reconstitution solution bottle and firmly attach the collar to the reconstitution solution bottle (Figure 5, Step 2).
      v. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial (Figure 5, Step 3).
      vi. Pick up the assembled bottles, and swirl the assembled bottles for at least 10 seconds (Figure 5, Step 4).
      vii. Wait for at least 30 minutes for the lyophilized reagent to go into solution.
      viii. After the lyophilized reagent has gone into solution, swirl the assembled bottles for at least 10 seconds and then slightly rock the solution within the glass vial back and forth to mix thoroughly.
   c. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle (Figure 5, Step 5).
   d. Carefully remove the reconstitution collar and glass vial (Figure 5, Step 6).
   e. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 5, Step 7).
   f. Discard the reconstitution collar and glass vial (Figure 5, Step 8).

Warning: *Avoid creating excessive foam when reconstituting reagents. Foam compromises the level-sensing by the Panther system.*

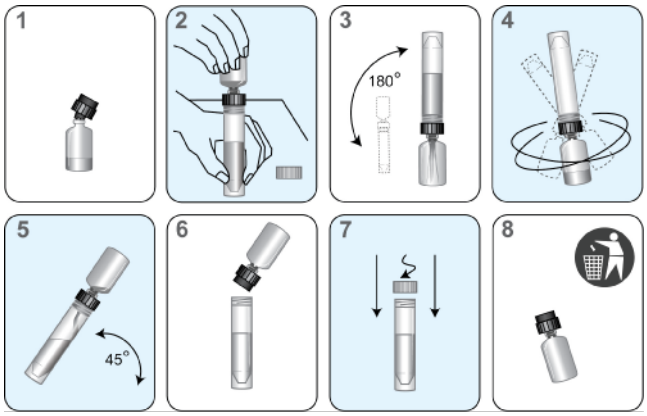


Figure 5. Reagent Reconstitution Process

D. Reagent Preparation for Previously Prepared Reagents

1. Remove the previously prepared reagents from storage (2°C to 8°C).
2. Previously prepared Amplification, Enzyme, Promoter reagents, and TCR must reach 15°C to 30°C prior to the start of the assay.
3. For previously prepared TCR, perform step C.1 above prior to loading on the system.
4. Swirl and invert the Amplification, Enzyme, and Promoter reagents to mix thoroughly prior to loading on the system. Avoid creating excessive foam when inverting reagents.
5. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

E. Specimen Handling

1. Ensure that processed specimens in primary tubes or undiluted specimens in secondary tubes have been stored properly per “Specimen Collection and Storage” on page 7.
2. Ensure frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
3. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther System* for additional onboard information.
4. Ensure that each primary collection tube contains up to 1200 µL of specimen or each SAT contains at least 700 µL of specimen. Refer to the table provided in *Specimen Collection* on page 7 to identify dead volume requirements for each primary and secondary tube type. If specimen dilution is necessary, see step E.6 below for additional information.
5. Just prior to loading specimens into a Sample Rack, centrifuge each specimen at 1000 to 3000*g* for 10 minutes. Do not remove caps. Bubbles in the tube can compromise the level-sensing by the Panther system.

   See *System Preparation*, step F.2 below, for information about loading the rack and removing the caps.

6. Dilute a plasma specimen 1:3 in a SAT or 1:100 in a secondary tube.

   A plasma specimen may be diluted in a secondary tube for testing on the Panther system.

⚠ Dilution of plasma specimens may only be used for quantitative results. Do not dilute plasma samples for diagnostic results.

Note: *If a specimen is diluted, it must be tested immediately after dilution.*

a. Dilution of low-volume specimens

   The volume of plasma specimens may be increased to the minimum volume required (700 µL) using Aptima Specimen Diluent. Specimens with at least 240 µL of plasma may be diluted with two parts specimen diluent (1:3) as follows:

   i. Place 240 µL of specimen in the SAT.

   ii. Add 480 µL of Aptima Specimen Diluent.

   iii. Cap the tube.

   iv. Gently invert 5 times to mix.

   Specimens diluted 1:3 can be tested using the 1:3 option on the Panther system (see the *Panther System Operator's Manual* for more information). The software will automatically report the neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

b. Dilution of high-titer specimens

   If a specimen's result is above the upper limit of quantitation, it may be diluted with 99 parts of Aptima Specimen Diluent (1:100) as follows:

   i. Place 30 µL of specimen in the SAT or a secondary tube.

   ii. Add 2970 µL of Aptima Specimen Diluent.

   iii. Cap the tube.

   iv. Gently invert 5 times to mix.

   Specimens diluted 1:100 can be tested using the 1:100 option on the Panther system (see *Panther System Operator's Manual* for more information). The software will automatically report the neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

   Note: *For diluted specimens with neat concentrations greater than the ULoQ, results will be reported using scientific notation.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther System Operator's Manual* and *Procedural Notes.* Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples into the Sample Rack. Perform the following steps for each sample tube (specimen, and, when necessary, calibrator and controls):
   a. Loosen one sample tube cap, but do not remove it yet.

      Note: *Be especially careful to avoid contamination by the spread of aerosols. Gently loosen caps on samples.*

   b. Load the sample tube into the Sample Rack.

   c. Repeat steps 2.a and 2.b for each remaining sample.

d. After the samples have been loaded into the Sample Rack, remove and discard each sample tube cap in one Sample Rack. To avoid contamination, do not pass a cap over any other Sample Racks or sample tubes.

e. If necessary, use a new, disposable transfer pipet to remove any bubbles or foam.

f. When the last cap has been removed, load the Sample Rack into the Sample Bay.

Note: *If running other assays and sample types at the same time, secure the Sample Retainer prior to loading the Sample Rack into the Sample Bay.*

g. Repeat steps 2.a to 2.f for the next Sample Rack.

Procedural Notes

A. Calibrator and Controls

1. The qHIV-1 positive calibrator, the qHIV-1 low positive control, qHIV-1 high positive control, and qHIV-1 negative control tubes can be loaded in any position in the Sample Rack and in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. The calibrator and controls are currently being processed by the system.
   b. Valid results for the calibrator and controls are registered on the system.
2. Once the calibrator and control tubes have been pipetted and are processing for the Aptima HIV-1 Quant Dx assay reagent kit, specimens can be tested with the associated, reconstituted kit for up to 24 hours **unless:**
   a. The calibrator or control results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. The calibrator and each control tube can be used once. Attempts to use the tube more than once can lead to processing errors.

B. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control

A run or specimen result may be invalidated by an operator if technical, operator, or instrument difficulties are observed while performing the assay and they are documented. In this case, specimens must be retested.

Assay Calibration

To generate valid results, an assay calibration must be completed. A single positive calibrator is run in triplicate each time a reagent kit is loaded on the Panther system. Once established, the calibration is valid for up to 24 hours. Software on the Panther system alerts the operator when a calibration is required. The operator scans a calibration coefficient found on the Master Lot Barcode Sheet provided with each reagent kit.

During processing, criteria for acceptance of the calibrator are automatically verified by the software on the Panther system. If less than two of the calibrator replicates is valid, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative control, of the low positive control, and of the high positive control must be tested each time a reagent kit is loaded on the Panther system. Once established, the controls are valid for up 24 hours. Software on the Panther system alerts the operator when controls are required.

During processing, criteria for acceptance of controls are automatically verified by software on the Panther system. To generate valid results, the negative control must give a result of “Not Detected” and the positive controls must give results within predefined parameters. If any one of the controls has an invalid result, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Internal Calibrator/Internal Control

Each sample contains an internal calibrator/internal control (IC). During processing, IC acceptance criteria are automatically verified by the Panther system software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested to obtain a valid result.

The Panther system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther System Operator's Manual.*

Interpretation of Results

Note: *Quantitative results of the Aptima HIV-1 Quant Dx assay has been evaluated with plasma. Serum may not be used to obtain quantitative results. Qualitative results have been evaluated with both plasma and serum.*

The Panther system automatically determines the concentration of HIV-1 RNA for specimens and controls by comparing the results to a calibration curve. HIV-1 RNA concentrations are reported in copies/mL and $\log_{10}$ copies/mL. The interpretation of results is provided in Table 1. If the 1:3 or 1:100 dilution is used for diluted specimens, the Panther system automatically calculates the HIV-1 concentration for the neat specimen by multiplying the the diluted concentration by the dilution factor and diluted samples are flagged as diluted.

Note: *For diluted specimens, results listed as "Not Detected" or "<30 detected" may be generated by diluting a specimen with a concentration above, but close to the LoD (limit of detection) or LLoQ (lower limit of quantitation). It is recommended to collect and test another neat specimen if a quantitative result is not obtained.*

The Panther system does not provide a qualitative result (i.e., "Reactive" or "Non-reactive") for diagnostic use. The operator must interpret the reported HIV-1 RNA concentration into a qualitative result (Table 1). Specimens with results listed as "Not Detected" are nonreactive for HIV-1 RNA. Specimens with results listed as "<30 detected" or specimens with results listed within the linear range indicate HIV-1 RNA was detected and these specimens are reactive for HIV-1 RNA.

Table 1: Result Interpretation

Reported Aptima HIV-1 Quant Dx Assay Result		HIV-1 RNA Concentration Interpretation	User's Diagnostic Qualitative Interpretation[c]
Copies /mL[a]	$\log_{10}$ Value[b]		
Not Detected	Not Detected	HIV-1 RNA not detected.	Non-reactive for HIV-1 RNA
<30 detected[e]	<1.47	HIV-1 RNA is detected but at a level below the LLoQ.	Reactive for HIV-1 RNA
30 to 10,000,000	1.47 to 7.00	HIV-1 RNA concentration is within the linear range of 30 to 10,000,000 copies/mL.	Reactive for HIV-1 RNA
>10,000,000	>7.00	HIV-1 RNA concentration is above the upper limit of quantitation (ULoQ).	Reactive for HIV-1 RNA
Invalid[d]	Invalid[d]	There was an error in the generation of the result. Specimen should be retested.	Invalid

[a] The conversion factor for copies to International Unit (IU) for the 3rd International Standard for HIV-1 RNA (10/152) is 0.35 copies/IU.
[b] Value is truncated to two decimal places.
[c] A diagnostic interpretation may be made from either serum or plasma specimens that have not been diluted.
[d] Invalid results are displayed in blue-colored font.
[e] The software's lowest reportable value is 30 copies/mL. The assay's highest LoD is 17.5 copies/mL for subtype G. For LoD values of all subtypes, see Table 3. The LoD using the WHO 3rd International Standard (subtype B) for HIV-1 RNA is 12.1 copies/mL (see Table 2).

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. This assay has been validated for use as a quantitative assay with only human EDTA and ACD plasma.

D. This assay has been validated for use as a qualitative assay with human EDTA and ACD plasma and serum.

E. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Aptima HIV-1 Quant Dx assay may result in underquantification of or failure to detect the virus.

Performance

Limit of Detection (LoD) Using the 3rd HIV-1 WHO International Standard

The limit of detection (LoD) is defined as the concentration of HIV-1 RNA that is detected at 95% or greater probability according to CLSI EP17-A2 (39). The LoD was determined by testing panels that consisted of dilutions of the 3rd HIV-1 WHO International Standard (subtype B, NIBSC code: 10/152) in HIV-1 negative plasma. Thirty replicates of each dilution were run on three Panther systems using three reagent lots for a total of 90 replicates for each dilution. Per CLSI EP17-A2, the results from the reagent lot with the highest concentration for the predicted detection limit are defined as LoD and are shown in Table 2. Through Probit analysis, the LoD for the Aptima HIV-1 Quant Dx assay is 12 copies/mL (35 IU/mL; 0.35 copies = 1 IU).

Table 2: Limit of Detection of the Aptima HIV-1 Quant Dx Assay Using the 3rd HIV-1 WHO International Standard

Predicted Detection Limit	Concentration (copies/mL)
10%	1.2
20%	1.6
30%	2.0
40%	2.5
50%	3.1
60%	3.8
70%	4.8
80%	6.2
90%	9.0
95%	12.1

Limit of Detection Across HIV-1 Subtypes and Groups

For HIV-1 group M (subtypes A, C, D, F, G, CRF01_AE, CRF02_AG) and groups N and O, seven panels were created by spiking either cultured HIV-1 virus or positive clinical specimens into HIV-1 negative human plasma (0 to 40 copies/mL). Each panel member was tested in 30 replicates with two reagent lots for a total of 60 replicates per panel member. Assignment of the concentration for clinical specimens or cultured virus stocks was determined using a comparator assay. Probit analysis was performed to generate 50% and 95% predicted detection limits. Per CLSI EP17-A2 (39), the results from the reagent lot with the highest concentration for the predicted detection limit are defined as LoD and are shown in Table 3.

Table 3: Limit of Detection across HIV-1 Subtypes and Groups

Subtype/Group	Predicted Detection Limit	Concentration (copies/mL)
A	50%	3.0
	95%	12.3
CRF01_AE	50%	1.8
	95%	6.2
CRF02_AG	50%	3.4
	95%	15.4
C	50%	2.0
	95%	10.7
D	50%	3.7
	95%	14.0
F	50%	2.1
	95%	8.3
G	50%	3.1
	95%	17.5
N	50%	1.2
	95%	7.8
O	50%	1.8
	95%	8.0

Linear Range

The linear range of the Aptima HIV-1 Quant Dx assay was established by testing panels that consisted of cultured HIV-1 subtype B virus diluted in HIV-1 negative human plasma according to CLSI EP06-A (40). Panels ranged in concentration from 1.30 to 7.30 log copies/mL. Testing was performed on seven Panther systems with two reagent lots of Aptima HIV-1 Quant Dx assay. As shown in Figure 6, the Aptima Quant Dx assay demonstrated linearity across the range tested.

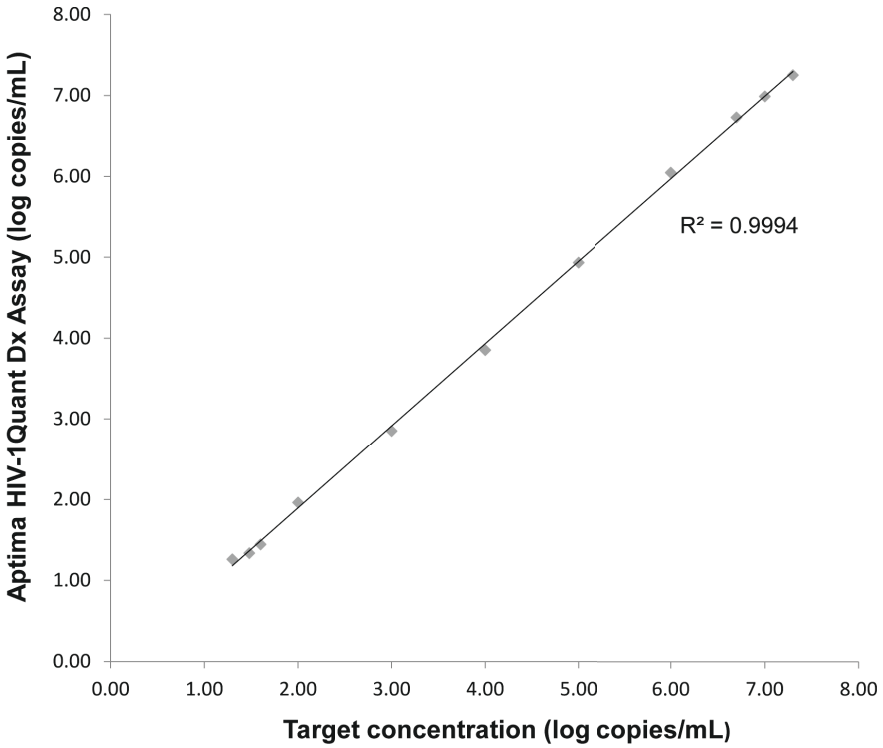


Figure 6. Linearity of the Aptima HIV-1 Quant Dx Assay

Linearity across HIV-1 Subtypes and Groups

The linear response of the Aptima HIV-1 Quant Dx assay across group M (subtypes A, B, C, D, F, G, H, CRF01_AE) and groups N and O was confirmed by testing panels that consisted of HIV-1 transcript diluted in buffer at concentrations ranging from 2.00 to 6.70 log copies/mL. Testing was performed on four Panther systems and six runs. Linearity was demonstrated across the range tested (Figure 7).

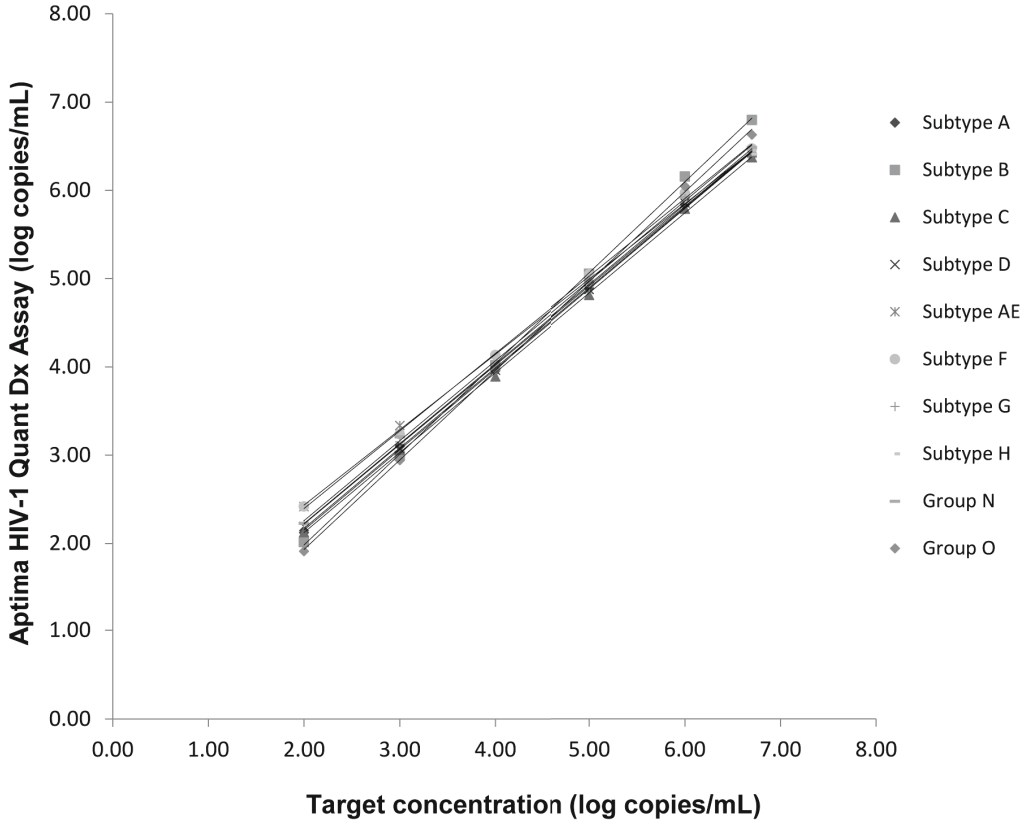


Figure 7. Linearity across Group M (Subtypes A, B, C, D, F, G, H, CRF01_AE) and Groups N and O

Lower Limit of Quantitation Using the 3rd HIV-1 WHO International Standard

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which HIV-1 RNA is reliably quantitated within a total error (TE), according to CLSI EP17-A2 (39). TE was calculated using the Westgard model (TE = |bias| + 2SD). To ensure accuracy and precision of measurements, the TE of the Aptima HIV-1 Quant Dx assay was set at 1 log copies/mL (i.e., at LLoQ, the difference between two measurements of more than 1 log copies/mL is statistically significant).

LLoQ was determined by testing panels that consisted of dilutions of the 3rd HIV-1 WHO International Standard (subtype B, NIBSC code: 10/152) in HIV-1 negative plasma. Per CLSI EP17-A2, panels were tested with three reagent lots in replicates of 30 for each lot from 23 runs. The results are shown in Table 4. The highest LLoQ for the three lots tested on the Aptima HIV-1 Quant Dx assay using the 3rd HIV-1 WHO International Standard is 15 copies/mL (1.17 log copies/mL; 42.9 IU/mL) (Table 5).

Table 4: Determination of LLoQ of the Aptima HIV-1 Quant Dx Assay Using the 3rd HIV-1 WHO International Standard

Reagent Lot	Target Concentration (log copies/mL)	Aptima HIV-1 Quant Dx (log copies/mL)	SD (log copies/mL)	|Bias| (log copies/mL)	Calculated TE (log copies/mL)
1	1.15	1.05	0.37	0.10	0.84
	1.24	0.94	0.35	0.30	1.00
	1.42	1.37	0.33	0.05	0.71
	1.54	1.47	0.22	0.07	0.50
	1.94	1.98	0.13	0.04	0.30
	2.42	2.45	0.07	0.03	0.17
2	1.15	0.50	0.33	0.65	1.31
	1.24	0.80	0.44	0.45	1.33
	1.42	0.93	0.37	0.49	1.24
	1.54	1.17	0.31	0.38	0.99
	1.94	1.75	0.21	0.19	0.62
	2.42	2.28	0.21	0.14	0.55
3	1.15	0.88	0.41	0.26	1.09
	1.24	0.98	0.35	0.27	0.97
	1.42	1.15	0.34	0.27	0.96
	1.54	1.35	0.37	0.20	0.93
	1.94	1.84	0.17	0.11	0.44
	2.42	2.37	0.11	0.05	0.27

SD=standard deviation

Table 5: Summary of LLoQ Using the 3rd HIV-1 WHO International Standard (3 Reagent Lots)

Reagent Lot	LLoQ (log copies/mL)	LLoQ (copies/mL)
1	0.94	8.7
2	1.17	15
3	0.98	9.5

Verification of LLoQ across HIV-1 Subtypes and Groups

LLoQ across HIV-1 subtypes and groups was verified following CLSI EP17-A2 (39). Panels were made for each HIV-1 group M (subtypes A, B, C, D, F, G, CRF01_AE, CRF02_AG), and groups N and O by spiking pooled HIV-1 negative human plasma with either naturally infected clinical samples or clinical isolates. Testing consisted of a total 30 replicates per panel member. The data in Table 6 shows the lowest concentration for each subtype or group at which TE was less than 1 log copies/mL. The highest LLoQ for all subtypes and groups tested was 30 copies/mL; this higher value, therefore, was selected as the LLoQ for the Aptima HIV-1 Quant Dx assay.

Table 6: Verification of LLoQ by HIV-1 Subtype or Group

Panel	LLoQ (copies/mL)
Subtype A	30
Subtype CRF01_AE	10
Subtype CRF02_AG	30
Subtype B	10
Subtype C	30
Subtype D	15
Subtype F	15
Subtype G	30
Group N	10
Group O	15

Precision

To assess precision of the Aptima HIV-1 Quant Dx assay, a panel that was made by spiking cultured HIV-1 subtype B virus into HIV-1 negative plasma was tested by three operators using three reagents lots on three Panther systems over 20 days (Table 7). The panel consisted of one HIV-1 negative panel member and eight HIV-1 positive panel members. Assignment of the concentration for clinical specimens or cultured virus stocks was determined using a comparator assay.

Table 7: Precision of the Aptima HIV-1 Quant Dx Assay

Number of Valid Replicates	Mean Concentration (log copies/mL)	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Run		Intra-Run		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
137	1.80	0.00	0.00	0.03	1.72	0.00	0.00	0.00	0.00	0.16	8.93	0.16	9.10
157	2.37	0.00	0.00	0.05	2.08	0.01	0.36	0.08	3.33	0.15	6.19	0.17	7.34
160	2.47[a]	0.00	0.00	0.03	1.37	0.03	1.35	0.07	2.97	0.12	5.03	0.15	6.15
162	2.95	0.00	0.00	0.08	2.57	0.02	0.61	0.10	3.29	0.09	3.04	0.15	5.20
162	3.80	0.01	0.32	0.03	0.80	0.02	0.48	0.06	1.49	0.07	1.80	0.10	2.53
159	4.93	0.00	0.00	0.02	0.37	0.04	0.77	0.05	1.10	0.04	0.71	0.08	1.56
162	5.69	0.00	0.00	0.02	0.27	0.04	0.66	0.03	0.58	0.07	1.29	0.09	1.58
162	6.71	0.00	0.00	0.01	0.22	0.04	0.52	0.04	0.60	0.05	0.78	0.08	1.13

CV=coefficient of variation, SD=standard deviation

[a]This panel member was diluted 1:3 with specimen diluent and tested to evaluate the precision of the diluted sample.

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD=0 and CV=0%. The total number of replicates tested was 162 for each panel; only replicates with a numerical value were analyzed.

Potentially Interfering Substances

The susceptibility of the Aptima HIV-1 Quant Dx assay to interference by elevated levels of endogenous substances and by drugs commonly prescribed to HIV-1 infected individuals was evaluated. HIV-1 negative human plasma samples and samples spiked to a concentration of 3 log copies/mL of HIV-1 RNA were tested.

No interference in performance of the Aptima HIV-1 Quant Dx assay was observed in the presence of albumin (90 mg/mL), hemoglobin (5 mg/mL), triglycerides (30 mg/mL), or unconjugated bilirubin (0.2 mg/mL).

No interference in performance of the Aptima HIV-1 Quant Dx assay was observed in the presence of the exogenous substances listed in Table 8 at concentrations at least three times the C_{max} (human plasma).

Table 8: Exogenous Substances

Exogenous Substance Pool	Exogenous Substances Tested
1	Lopinavir, indinavir, saquinavir, ritonavir, nelfinavir mesylate, darunavir, amprenavir, atazanavir
2	Nevirapine, efavirenz, rilpivirine, clarithromycin, amphotericin B
3	Tenofovir disoproxil fumarate, adefovir dipivoxil, ribavirin, enfuvirtide, maraviroc, raltegravir, dolutegravir
4	Abacavir sulfate, didanosine, zidovudine, lamivudine, stavudine, entecavir, telbivudine, emtricitabine
5	Paroxetine HCl, fluoxetine, sertraline
6	Ganciclovir, valacyclovir, acyclovir, rifampin/rifampicin, ethambutol
7	Ciprofloxacin, azithromycin, amoxicillin, cephalexin, ampicillin, trimethoprim
8	Valganciclovir hydrochloride, boceprevir, telaprevir, simeprevir, sofosbuvir
9	Pegylated interferon alpha -2b, interferon alpha -2a, interferon alpha -2b
10	Heparin, EDTA, sodium citrate
11	Tipranavir
12	Isoniazid

Clinical plasma specimens listed in Table 9 from patients with elevated levels of defined substances or from patients with the diseases listed were tested with the Aptima HIV-1 Quant Dx assay with and without the presence of 3 log copies of HIV-1 RNA. No interference in performance was observed.

Table 9: Tested Clinical Specimen Types

	Clinical Specimen Types
1	Rheumatoid factor (RF)
2	Antinuclear antibody (ANA)
3	Anti-Jo-1 antibody (JO-1)
4	Systemic lupuserythematosus (SLE)
5	Rheumatoid arthritis (RA)
6	Multiple sclerosis (MS)
7	Hyperglobulinemia)
8	Elevated alanine aminotransferase (ALT)
9	Alcoholic cirrhosis (AC)
10	Multiple myeloma (MM)
11	Lipemic (elevated lipid)
12	Icteric (elevated bilirubin)
13	Hemolyzed (elevated hemoglobin)
14	Elevated protein albumin
15	HCV antibodies
16	HBV antibodies
17	HIV-2 antibodies

Specificity

Specificity of the Aptima HIV-1 Quant Dx assay was determined using 120 fresh and 510 frozen HIV-1 negative plasma specimens, and using 120 fresh and 510 frozen HIV-1 negative serum specimens. All results were non-reactive (specificity of 100%; 95% CI: 99.4-100%).

Table 10: Specificity in Plasma and Serum Specimens

	Fresh Plasma	Frozen Plasma	Plasma Total	Fresh Serum	Frozen Serum	Serum Total
Valid replicates (n)	120	510	630	120	510	630
Non-Reactive	120	510	630	120	510	630
Specificity (95% CI)	100% (97.0-100)	100% (99.3-100)	100% (99.4-100)	100% (97.0-100)	100% (99.3-100)	100% (99.4-100)

CI=confidence interval

Analytical Specificity

Potential cross-reactivity to pathogens (Table 11) was evaluated in the Aptima HIV-1 Quant Dx assay in the presence or absence of 3 log copies/mL HIV-1 RNA in HIV-1 negative plasma. No interference in the performance of the assay was observed in the presence of the pathogens.

Table 11: Pathogens Tested for Analytical Specificity

Pathogen	Concentration
Hepatitis A virus	100,000 PFU/mL[a]
Hepatitis B virus	100,000 IU/mL[b]
Hepatitis C virus	100,000 IU/mL
Hepatitis G virus	100,000 copies/mL
Herpes simplex virus 1 (HSV-1)	100,000 PFU/mL
Herpes simplex virus 2 (HSV-2)	75,000 PFU/mL
Human herpes virus 6	100,000 copies/mL
Human herpes virus 8	42,000 PFU/mL
HIV-2	5,500 PFU/mL
Human T-cell lymphotropic virus (HTLV)	100,000 vp/mL[c]
West Nile virus	100,000 copies/mL
Parvovirus B19	100,000 IU/mL
Cytomegalovirus	100,000 copies/mL
Epstein-Barr virus	100,000 copies/mL
Adenovirus type 5	100,000 PFU/mL
Dengue virus	100,000 copies/mL
Influenza A virus	100,000 PFU/mL
Staphylococcus aureus	1,000,000 CFU/mL[d]
Propionibacterium acnes	1,000,000 CFU/mL
Staphylococcus epidermidis	1,000,000 CFU/mL
Neisseria gonorrhoeae	1,000,000 CFU/mL
Chlamydia trachomatis	300,000 IFU/mL[e]
Candida albicans	1,000,000 CFU/mL

[a]PFU/mL = Plaque forming units per mL.
[b]IU/mL = International units per mL.
[c]vp/mL = Viral particles per mL.
[d]CFU/mL = Colony forming units per mL.
[e]IFU/mL = Inclusion forming units per mL.

Repeatability of Clinical Specimens

Ten clinical plasma samples were tested in three replicates using the Aptima HIV-1 Quant Dx assay. The average concentration and standard deviation is shown in Table 12.

Table 12: Repeatability of Clinical Specimens

Specimen	Average Concentration (log copies/mL)	SD
1	2.57	0.06
2	3.20	0.03
3	3.24	0.06
4	3.97	0.02
5	4.20	0.05
6	4.85	0.01
7	5.17	0.04
8	5.51	0.06
9	5.84	0.02
10	6.64	0.00

Sample Dilution Using Specimen Diluent

To assess sample dilution, a panel that consisted of 11 samples with concentrations that spanned the linear range of the Aptima HIV-1 Quant Dx assay and that consisted of two samples above the upper limit of quantitation of the assay were tested neat and diluted (1:3 or 1:100 in specimen diluent) in triplicate (Table 13).

Table 13: Sample Dilution

Dilution	Average Neat Concentration (log copies/mL)	Average Reported Concentration[a] (log copies/mL)	Difference
1:3	2.57	2.72	0.15
	3.20	3.33	0.13
	3.24	3.55	0.30
	3.97	4.05	0.07
	4.20	4.24	0.04
	4.85	4.81	-0.04
	5.17	5.08	-0.08
	5.51	5.32	-0.19
	5.84	5.94	0.10
	6.64	6.66	0.02
	2.46[b]	2.19	-0.27
1:100	>7.00 (7.16[c])	7.48	0.32
1:100	>7.00 (7.40[c])[b]	7.39	-0.01

[a]Reported concentration is the value reported by the Panther system after the dilution factor has been applied
[b]Spiked specimen
[c] All results > 7.00 log copies/mL were estimated using additional analysis

Method Correlation

The performance of the Aptima HIV-1 Quant Dx assay was assessed against a CE-marked comparator assay by testing undiluted clinical plasma specimens from HIV-1 infected patients on four Panther systems with two reagent lots. A total of 342 frozen and 108 fresh plasma specimens with quantifiable results in both the Aptima HIV-1 Quant Dx assay and the comparator assay were used for the linear regression (Figure 8). The specimens included HIV-1 group M (subtypes A, B, C, D, F, G, H, CRF01_AE, CRF02_AG).

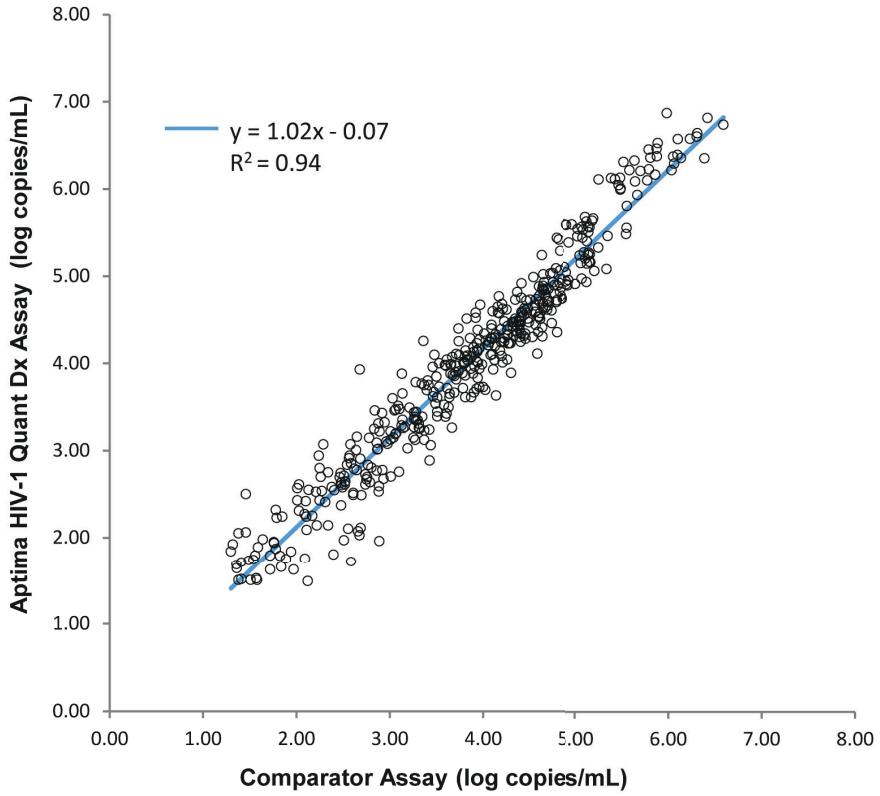


Figure 8. Correlation between the Aptima HIV-1 Quant Dx Assay and Comparator Assay

Diagnostic Agreement

To assess diagnostic agreement, specimens from HIV-1 positive individuals were tested using the Aptima HIV-1 Quant Dx assay and a comparator CE-marked HIV-1 qualitative assay: 414 specimens had valid results (Table 14). The results for both assays were categorized as follows. Any result giving a quantifiable or detectable result was categorized as “Detected.” Any result of target not detected was categorized as “Target Not Detected.”

Table 14: Diagnostic Agreement between Aptima HIV-1 Quant Dx Assay and Comparator Assay

		Aptima HIV-1 Quant Dx Assay	
		Detected	**Target Not Detected**
Comparator Assay	**Detected**	214	0
	Target Not Detected	0	200

Carryover

To establish that the Panther system minimizes the risk of false positive results arising from carryover contamination, a multi-run analytical study was conducted using spiked panels on two Panther systems. Carryover was assessed using high titer HIV-1 spiked samples (7 log copies/mL) interspersed between HIV-1 negative samples in a checkerboard pattern. Testing was carried out over five runs. The overall carryover rate was 0% (n=469).

Seroconversion Panel

Nineteen HIV-1 seroconversion panel sets, consisting of 204 samples, were tested using the Aptima HIV-1 Quant Dx assay. Detection of HIV-1 RNA was compared to detection with p24 antigen tests and with HIV-1/2 antibody tests. The number of days to the first reactive result using p24 antigen tests, anti-HIV 1/2 antibody tests, and the Aptima HIV-1 Quant Dx assay is listed in Table 15. The Aptima HIV-1 Quant Dx assay detected HIV-1 RNA an average of 5.58 and 11.16 days before p24 antigen and anti-HIV 1/2 antibody tests.

Table 15: Seroconversion Panel Data Summary

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members			Days to First Reactive Result			Difference in Days to First Reactive Result (Based on Bleed Date)	
		Aptima HIV-1 Quant Dx	HIV p24 Antigen	Anti-HIV 1/2 Antibody	Aptima HIV-1 Quant Dx	HIV p24 Antigen	Anti-HIV 1/2 Antibody	Days Earlier Detection Than HIV p24 Antigen	Days Earlier Detection Than Anti-HIV 1/2 Antibody
6248	7	3	2	1	14	18	25	4	11
6243	10	6	3	2	18	25	32	7	14
6247	9	4	4	1	21	21	30	0	9
9016	10	3	2	0	27	30	34[a]	3	7
9018	11	5	3	2	21	28	32	7	11
9020	22	5	4	1	83	87	97	4	14
9021	17	5	4	1	43	47	57	4	14
9022	9	3	2	1	23	25	32	2	9
9023	22	5	3	0	71	78	85[a]	7	14
9030	16	5	3	1	40	47	54	7	14
9034	13	4	3	1	41	46	53	5	12
9089	6	5	3	2	7	16	20	9	13
12008	13	7	4	4	21	28	33	7	12
PRB962	6	4	2	0	7	14	17[a]	7	10
PRB963	7	4	2	0	9	17	21[a]	8	12
PRB966	10	5	3	2	35	44	48	9	13
PRB974[b]	4	3	2	1	7	9	16	2	9
PRB975[b]	5	3	1	0	7	14	14[a]	7	7
PRB978[b]	7	3	1	0	26	33	33[a]	7	7
Total	**204**	**82**	**51**	**20**			**Mean**	**5.58**	**11.16**
							Median	**7**	**12**

[a]All bleeds in this panel were non-reactive for Anti-HIV 1/2 Antibody. The last bleed day was used as the "Days to First Reactive Result."
Anti-HIV-1/2 Antibody testing was completed with Abbott Anti-HIV 1/2, with the following exceptions:
[b]Panels PRB974, PRB975, and PRB978 were tested with Siemens Anti-HIV 1/2 test.
HIV-1 p24 Antigen testing was completed with Coulter HIV-1 p24 Ag, with the following exceptions:
[b]Panels PRB974, PRB975, and PRB978 were tested with BioMerieux p24 Ag test.

Serum, Plasma Equivalency Study

To assess equivalency, matched sets of serum and plasma (25 HIV-1 positive and 25 HIV-1 negative), and 40 samples that were spiked with cultured HIV-1 (50-1,000,000 copies/mL in HIV-1 negative plasma and serum) were tested with the Aptima HIV-1 Quant Dx assay. The negative agreement was 100.0% (95% CI: 97.0%-100.0%). The positive agreement was 98.4% (95% CI: 95.4%-99.5%).

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AW-11853-001 Rev. 010
2020-11

2.2 Aptima DBS Extraction Buffer

Aptima™ DBS Extraction Buffer

For *in vitro* diagnostic use.

For US export only.

Intended Use

The Aptima DBS Extraction Buffer is intended for extraction of HIV viral nucleic acid from Dried Blood Spot (DBS) specimens for testing with an Aptima HIV-1 Quant Dx assay (PRD-03000).

Principles of the Procedure

Dried blood spots are added in the Aptima Specimen Aliquot Tubes (SATs) containing Aptima DBS Extraction Buffer. During the incubation step, viral nucleic acid is released from the dried blood spot. Refer to the appropriate assay package insert for instructions on preparation and handling of the DBS specimen types.

Reagents

Aptima DBS Extraction Buffer, Cat. No. PRD-04772

(**store at 15°C to 40°C upon receipt)**

Symbol	Component	Quantity
DEB	**DBS Extraction Buffer** *Phosphate Buffered solution containing detergent.*	1 X 104 mL

Materials Required But Available Separately

Note: *Materials available from Hologic have catalog numbers listed.*

Note: *Aptima Specimen Aliquot Tubes and Transport Tube Caps are for general laboratory use and are not specifically intended for a particular IVD test*

Material	Cat. No.
Aptima Specimen Aliquot Tubes (100 pack)	503762
Transport Tube Caps (100 pack)	504415
Calibrated pipettors	—
Aerosol-barrier pipette tips	—

Warnings and Precautions

Note: *Hazard Communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

A. For *in vitro* diagnostic use.

B. Do not use Aptima Specimen Diluent or other buffers to extract DBS specimens.

C. Use only supplied or specified disposable laboratory ware.

D. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

E. Take care to avoid cross-contamination during the specimen handling steps. Specimens can contain high levels of target. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. If gloves come in contact with specimen, change gloves to avoid cross-contamination.

F. Do not use this buffer after its expiration date.

Storage and Handling Requirements

Store Aptima DBS Extraction Buffer at 15°C to 40°C. Once an extraction buffer bottle is opened, it can be used for up to 30 days. Cap the extraction buffer bottle immediately after use.

Specimen Extraction and Test Procedure

Refer to the appropriate assay package insert for extraction and test procedures.


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AW-17270-001, Rev. 001
2018-09

2.3 Dried Blood Spot (DBS) Supplement to the Aptima HIV-1 Quant Dx Assay

Dried Blood Spot (DBS) Supplement to the Aptima™ HIV-1 Quant Dx Assay

For *in vitro* diagnostic use.

For US export only.

General Information

Introduction

This package insert is a supplement to the *Aptima HIV-1 Quant Dx Assay* package insert (AW-11853). This document provides explanations, warnings and precautions, and instructions for preparation and testing of the Dried Blood Spot (DBS) sample type on the Aptima HIV-1 Quant Dx assay for HIV-1 viral load (VL) monitoring and early infant diagnosis (EID). For general warnings and precautions, as well as reagent preparation on the Aptima HIV-1 Quant Dx assay, refer to AW-11853.

Intended Use

The Aptima HIV-1 Quant Dx assay is an *in vitro* nucleic acid amplification test for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) RNA groups M, N, and O on the fully automated Panther™ system. It is intended for use as an aid in the diagnosis of HIV-1 infection, as a confirmation of HIV-1 infection, and as an aid in clinical management of patients infected with HIV-1.

In addition, the Aptima HIV-1 Quant Dx assay may be used as an aid in the diagnosis of acute or primary HIV-1 infection. Presence of HIV-1 RNA in the plasma, serum, or blood of patients without antibodies to HIV-1 is indicative of acute or primary HIV-1 infection. The Aptima HIV-1 Quant Dx assay may be used as a supplemental test for specimens that have repeat reactive results with approved HIV immunoassays. If the specimen is reactive in the Aptima HIV-1 Quant Dx assay, HIV-1 infection is confirmed.

The Aptima HIV-1 Quant Dx assay may also be used in conjunction with clinical presentation and other laboratory markers for disease prognosis in HIV-1 infected individuals. The Aptima HIV-1 Quant Dx assay may also be used as an aid in EID of HIV-1 infection in infants below 18 months of age using DBS. The Aptima HIV-1 Quant Dx assay may be used as an aid in monitoring the effect of antiretroviral treatment by measuring changes in the concentration of HIV-1 RNA in plasma and DBS samples.

When the Aptima HIV-1 Quant Dx assay is used as an aid in the diagnosis of HIV-1 infection, performance for qualitative results is established with both plasma and serum specimens as well as DBS samples from infants below 18 months of age. When used as an aid in monitoring the effect of antiretroviral therapy, performance for quantitative results is established with plasma and DBS specimens only. Serum specimens may not be used for quantitative results.

This assay is not intended for use in screening blood or plasma donors.

Summary and Explanation of the Test for the DBS Sample

DBS specimens may be used for viral load monitoring and for detecting virological failure at a 1000 copies/mL cut-off. (1) DBS specimens may also be used as an aid in the EID of infection with HIV-1 in infants below 18 months of age. (2).

Infants infected with HIV are at a high risk of death in the first year of life and timely initiation of anti-retroviral treatment (ART) reduces morbidity and mortality significantly. Serological HIV testing for EID is not recommended due to maternal IgG antibodies that can transfer across the placenta and persist in an uninfected infant up to 18 months of age, potentially leading to false positive HIV antibody test results. For diagnosis of infection with HIV-1 in children below

18 months of age, assays that detect components of the HIV-1 virus, such as HIV-1 RNA or p24 antigen are required. WHO recommends virological testing of infants below 18 months of age using HIV-1 DNA assays, HIV-1 RNA assays, or HIV-1 p24 antigen testing. DBS is the recommended sample type for EID when using HIV RNA detection methods. (2,3)

Warnings and Precautions

Note: *Hazard Communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

A. For *in vitro* diagnostic use.

B. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther System Operator's Manual* prior to performing this assay.

DBS Specimen Related

C. Specimens may be infectious. Use Universal Precautions (4,5,6) when performing this assay. Proper handling and disposal methods should be established according to local regulations.(6) Only personnel adequately trained in the use of the Aptima HIV-1 Quant Dx assay and trained in handling infectious materials should perform this procedure.

D. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

E. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens and when processing DBS samples. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

F. Collect and handle venous blood (EDTA) and finger stick or heel stick blood used to prepare DBS as well as DBS cards according to local guidelines for prevention of bloodborne pathogens transmission.

G. It is recommended to prepare at least three DBS spots on each DBS card.

H. Inappropriate preparation, drying, storage and handling of DBS may lead to inaccurate test results.

I. Ensure that DBS cards are fully dried prior to storage in zip lock bags with desiccant. Insufficiently dried DBS samples may have decreased stability and may lead to inaccurate results.

J. Ensure that unused DBS cards are stored and handled according to DBS card manufacturer's instructions.

K. For additional details on DBS preparation and handling, refer to DBS card manufacturer's instructions.

L. To avoid carry-over contamination, ensure that tools used for cutting and handling of the circles containing the dried blood are decontaminated prior and after contact with the sample.

M. Only use Aptima DBS Extraction Buffer for extraction of DBS samples. Do not use Aptima Specimen Diluent or other buffers to extract DBS specimens.

N. For additional laboratory-related warnings and precautions, refer to the *Aptima HIV-1 Quant Dx Assay* package insert (AW-11853).

Assay Related

O. Quantitative results of the Aptima HIV-1 Quant Dx assay have been evaluated with DBS and plasma. Serum may not be used to obtain quantitative results. Qualitative results for plasma, serum, and DBS have been evaluated. Do not use the reagent kit, the calibrator, or the controls after the expiration date.

P. Do not use DBS cards after expiration date specified by the manufacturer. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Assay fluids can be from different lot numbers. Controls and the calibrator can be from different lot numbers.

Q. Avoid microbial and nuclease contamination of reagents.

R. Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Panther System Test Procedure* for more information.

S. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

T. For hazard communication information, refer to the *Aptima HIV-1 Quant Dx Assay* package insert (AW-11853).

Specimen Collection and Storage for DBS

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

Whole blood specimens collected in EDTA or capillary blood collected by finger or heel stick may be used.

A. Specimen Collection and Preparation of DBS

- Whole blood collected in appropriate collection tubes may be stored for up to 24 hours at 2°C to 30°C prior to addition to the DBS cards. Prior to adding to the DBS card, mix blood thoroughly. Capillary blood may be collected using finger or heel stick according to standard procedure and local practice.
- Add approximately 70 µL of whole blood to the center of the one-half inch (12 millimeter) circles of the Ahlstrom/Munktel TFN cards or equivalent, (for example, Whatman 903). If finger stick or heel stick blood is used, add approximately 3-5 drops (approximately 70 µL) to each circle, ensuring that the entire surface of the circle (both sides of the DBS card) is saturated.

- Air dry DBS cards at ambient temperature (15°C to 30°C) for 4 to 24 hours. Take care to ensure DBS cards are kept away from direct sunlight, do not touch each other, and are fully dried prior to packing, storage, and shipment.

  Note: *DBS prepared with insufficient blood, insufficient drying, and/or inappropriate handling or storage of DBS cards may lead to inaccurate test results.*

B. DBS Specimens

For up to 24 hours after specimen collection, primary collection tubes containing whole blood may be stored at 2°C to 30°C, prior to preparing DBS (Figure 1, upper box).

Prepared DBS may be stored under one of the following conditions (Figure 1, lower boxes):

- DBS card at 2°C to 30°C for up to 12 weeks at ambient humidity, or
- DBS card at -15°C to -35°C for up to 12 weeks, or
- DBS card at 40°C for up to 2 weeks at 85% humidity.

Prior to testing, extracted DBS in SATs may be stored at 15°C to 30°C for up to 24 hours.

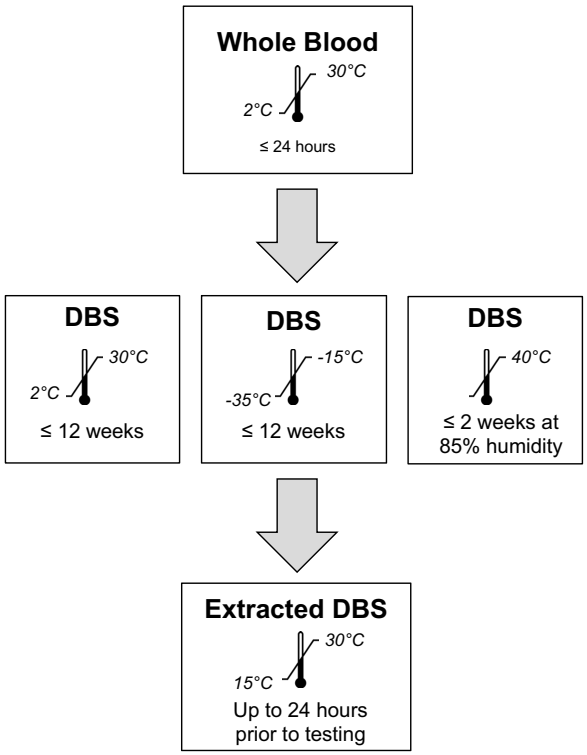

Figure 1. Storage Conditions for DBS

Samples Onboard the Panther System

Extracted DBS may be left on the Panther system uncapped for up to a total of 8 hours. Samples may be removed from the Panther system and tested as long as the total time onboard does not exceed 8 hours prior to the pipetting of the sample by the Panther system.

Specimen Transport

Maintain sample storage conditions as described in *Specimen Collection and Storage for DBS*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima HIV-1 Quant Dx assay for use on the Panther system are provided in the *Aptima HIV-1 Quant Dx Assay* package insert (AW-11853).

Materials Required But Available Separately for DBS Sample Type

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Aptima DBS Extraction Buffer (100 mL)	PRD-04772
Aptima SATs (100 pack)	503762
Transport Tube Cap (100 pack) *Cap for SAT*	504415
Commercially available DBS cards: *Ahlstrom/Munktel TFN cards or equivalent (e.g.,Whatman 903)*	—
Scissors, forceps or other tools to release the DBS spot from the DBS card.	—
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—
Reagent replacement caps *Amplification, Enzyme, and Promoter reagent reconstitution bottles* *TCR bottle*	 *CL0041 (100 caps)* *CL0040 (100 caps)*
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tube (ACD, EDTA, PPT) options: *13 mm x 100 mm* *13 mm x 75 mm* *16 mm x 100 mm*	 — — —
Centrifuge	—
Tube rocker	—

Panther System Test Procedure

A. Extraction of DBS Specimens

1. Allow specimens to reach 15°C to 30°C prior to processing.
2. Add 1 mL of DBS Extraction Buffer into the SAT.
3. Using a decontaminated tool (i.e., pipette tip, forceps, or scissors), transfer the DBS specimen into a SAT containing the DBS Extraction Buffer. Each DBS specimen should be approximately 12 mm in diameter.

   Note: *For non-perforated DBS cards, ensure the DBS specimen adheres to the side of the SAT.*
4. Close the SATs containing the DBS Extraction Buffer and DBS completely, using Transport Tube caps.
5. Rock gently at ambient temperature for 30 minutes. Ensure the DBS Extraction Buffer washes over the DBS specimen during rocking. Avoid creating excessive foam.

   Note: *Extracted DBS in the SAT may be stored up to 24 hours at 15°C to 30°C prior to testing.*
6. Prior to loading on the Panther system, centrifuge SAT containing the extracted DBS for 2 minutes at 3,000 g.
7. Load SAT containing the DBS on the Panther system (extracted DBS may be stored on the Panther system for up to 8 hours).

Note: *To avoid carry-over contamination, ensure tools used for sample preparation and handling are decontaminated in between multiple samples.*

Note: *The 8 hours of on-board time is not additive to the 24 hour storage period after extraction.*

B. System Preparation for DBS Specimens

1. Set up the system according to the instructions in the *Panther System Operator's Manual.*
2. Load specimen rack.
3. **Apply Dried Blood Spot Conversion Factor** to assay test orders for DBS specimens.

To apply the Dried Blood Spot Conversion Factor to an entire rack of DBS specimens:

a. From the *Sample Rack Loading* screen, select **Apply Dilution All**.

The *Dilution Factor* window appears.

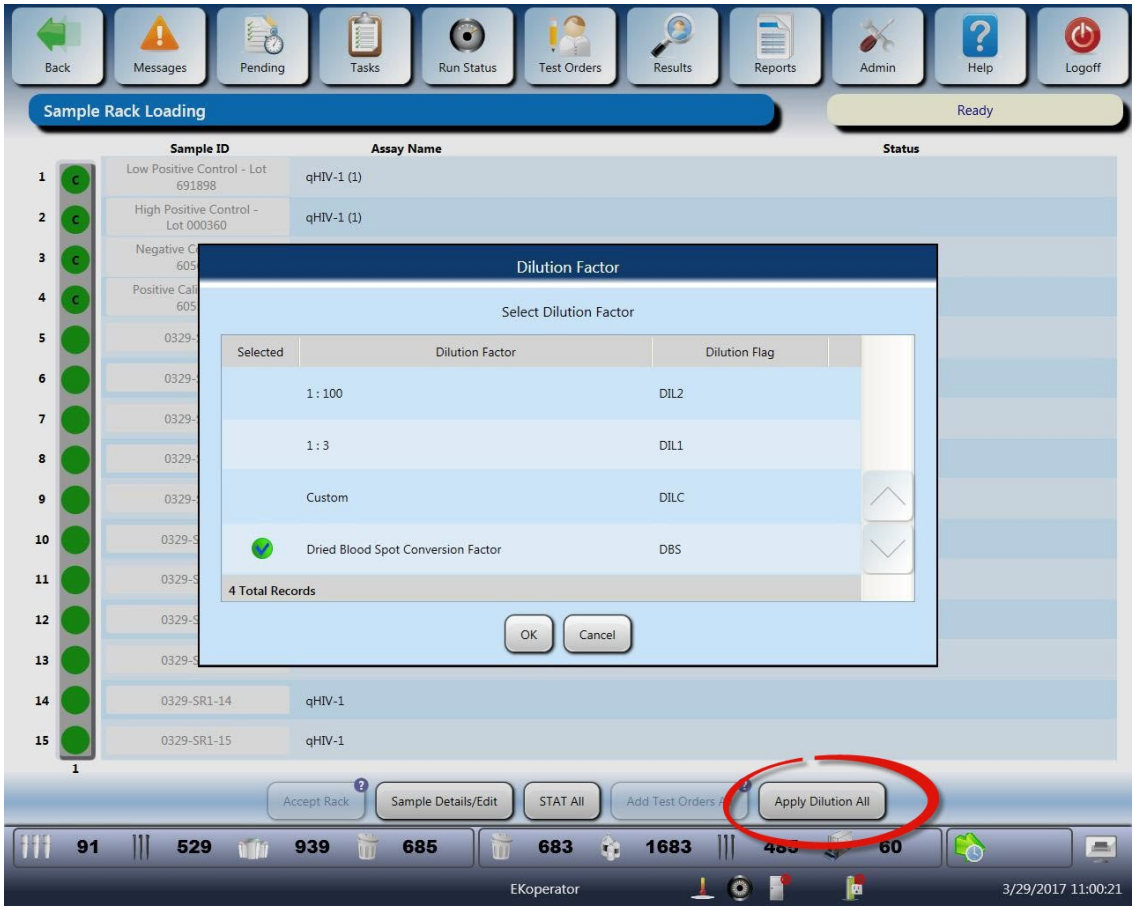

Figure 2. The Dilution Factor Window in the Sample Rack Loading Screen

b. Select **Dried Blood Spot Conversion Factor**.

c. Select **OK.**

A *Set Dilution Factor for Rack* window appears.

d. Select **Yes** to apply the Dried Blood Spot Conversion Factor flag to the entire rack of DBS specimens.

To apply the Dried Blood Spot Conversion Factor to a single test order (for example, third sample in rack, see illustration below):

a. From the *Sample Details* screen, select the test order to be processed and select **Apply Dilution**.

The *Dilution Factor* window appears.

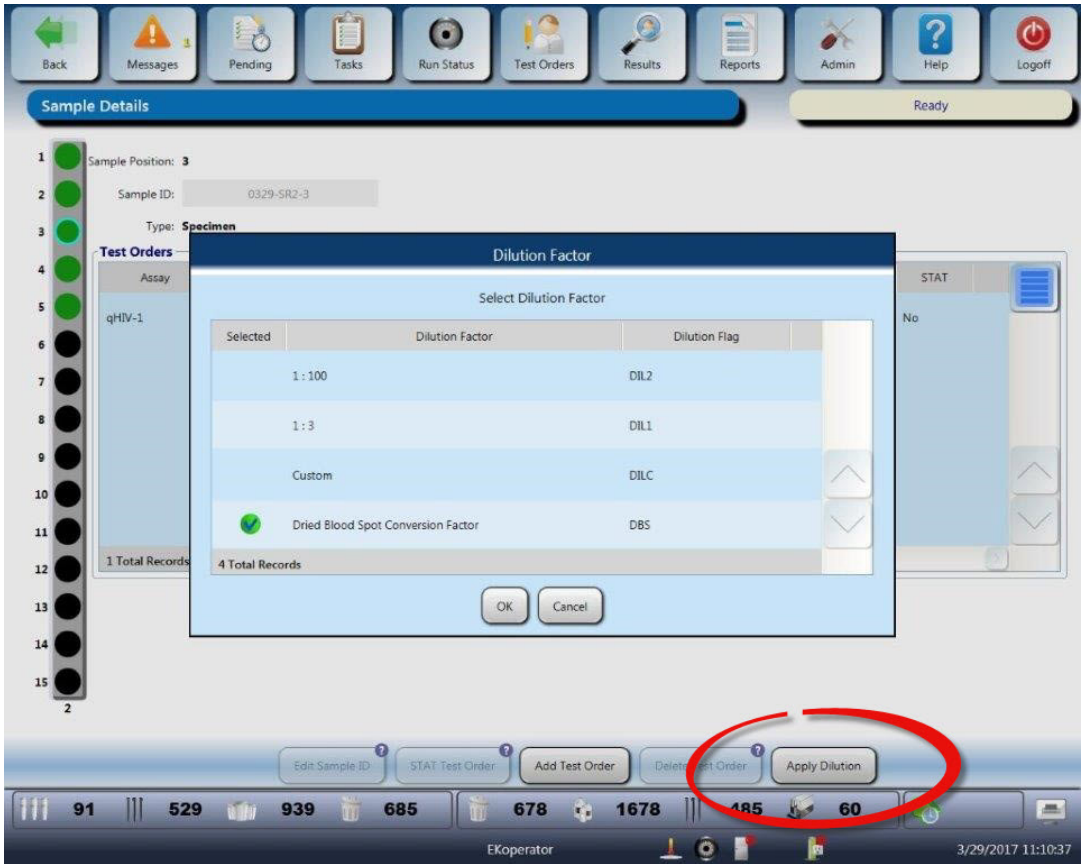

Figure 3. The Dilution Factor Window in the Sample Details Screen

b. Select **Dried Blood Spot Conversion Factor**.

c. Select **OK** to apply the Dried Blood Spot Conversion Factor flag to all selected test orders.

If necessary, the Dried Blood Spot Conversion Factor can be removed from test orders prior to the start of processing.

To delete the Dried Blood Spot Conversion Factor from an entire rack:

1. From the *Sample Rack Bay* screen, double-click the loaded rack of interest.

   The *Sample Rack Loading* screen appears for the selected rack.

2. Select **Apply Dilution All**.
3. From the *Dilution Factor* window, de-select **Dried Blood Spot Conversion Factor**.
4. Select **OK.**

   A *Set Dilution Factor for Rack* window appears.

5. Select **Yes** to delete the Dried Blood Spot Conversion Factor from an entire rack.

To delete the Dried Blood Spot Conversion Factor assay test orders:

1. From the *Sample Rack Bay* screen, double-click the loaded rack with the specimen(s) of interest.

   The *Sample Rack Loading* screen appears for the selected sample rack.
2. From the *Sample Rack Loading* screen, double-click the specimen of interest.

   The *Sample Details* screen appears with the current test orders for the selected specimen.
3. Select the test order of interest from the *Test Orders* panel.
4. Select **Apply Dilution.**
5. From the *Dilution Factor* window, de-select **Dried Blood Spot Conversion Factor**.
6. Select **OK** to delete the Dried Blood Spot Conversion Factor from the test order.

Procedural Notes for Calibrators and Controls

For DBS samples, no DBS positive and negative controls are provided. DBS samples require the same calibrators and controls used for serum and plasma sample types. Refer to AW-11853.

Quality Control

A run or specimen result may be invalidated by an operator if technical, operator, or instrument difficulties are observed while performing the assay and are documented. In this case, specimens must be retested.

Assay Calibration

DBS samples require the same calibrators used for serum and plasma sample types. Refer to AW-11853.

Negative and Positive Controls

DBS samples require the same controls used for serum and plasma sample types. Refer to AW-11853.

Internal Calibrator/Internal Control

Each sample contains an internal calibrator/internal control (IC). Refer to AW-11853.

Interpretation of Results for DBS

The Panther system automatically determines the concentration of HIV-1 RNA for specimens and controls by comparing the results to a calibration curve. For DBS specimens tested, the Panther system automatically reports copies/mL and log_{10} copies/mL of HIV-1 RNA based on the DBS Conversion factor. The log conversion for DBS LoD of 883 c/mL is 2.95 log c/mL. The viral load interpretation of results is provided in Table 1.

The Panther system does not provide a qualitative result (i.e., “Reactive” or “Non-reactive”) for diagnostic use (EID). The operator must interpret the reported HIV-1 RNA concentration into a qualitative result (see Table 2). Specimens with results listed as “Not Detected” are nonreactive for HIV-1 RNA. Specimens with results listed as “<883 detected” or specimens with results listed within the linear range indicate HIV-1 RNA was detected and these specimens are reactive for HIV-1 RNA. Specimens with results <1900 copies/mL should be retested to confirm reactivity for HIV diagnosis.

Table 1: Results Interpretation for DBS Viral Load Specimens

Reported Aptima HIV-1 Quant Dx Assay Result		HIV-1 RNA Concentration Interpretation
Copies /mL	**Log_{10} Value[a]**	
Not Detected	Not Detected	HIV-1 RNA not detected.
<883 detected	<2.95	HIV-1 RNA is detected but at a level below the lower limit of quantitation for DBS (LLoQ DBS 883 copies/mL)
883 to 10,000,000	2.95 to 7.00	HIV-1 RNA concentration is within the linear range of the assay for DBS (883 copies/mL to 10,000,000)
>10,000,000	>7.00	HIV-1 RNA concentration is above the upper limit of quantitation (ULoQ).
Invalid[c]	Invalid[c]	There was an error in the generation of the result. Specimen should be retested.

[a] Value is truncated to two decimal places.

[b] Invalid results are displayed in blue-colored font.

Table 2: Results Interpretation for DBS Diagnostic Specimens

Reported Aptima HIV-1 Quant Dx Assay Result		User's Diagnostic Qualitative Interpretation
Copies /mL	**Log_{10} Value[a]**	
Not Detected	Not Detected	HIV-1 RNA not detected.
<883 detected or 883 to 1900	<2.95 or 2.95 to 3.28	Retest to confirm reactive diagnostic results. Only confirmed positives are considered reactive.[b]
1901 to 10,000,000	3.28 to 7.00	Reactive for HIV-1 RNA
>10,000,000	>7.00	Reactive for HIV-1 RNA
Invalid[c]	Invalid[c]	There was an error in the generation of the result. Specimen should be retested.

[a] Value is truncated to two decimal places.

[b] **World Health Organization, Policy Brief.** July 2018. Update on Antiretroviral Regimens for Treating and Preventing HIV Infection and Update on Early Infant Diagnosis. HIV Treatment—Interim Guidance. Geneva, Switzerland..

[c] Invalid results are displayed in blue-colored font.

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Ensure that use of this assay is with Panther System Software Version 6.2 or higher.

C. Different test methodologies may give different reported values. To reduce risk of misinterpretation of results when transitioning to a new assay, it is recommended that new methodologies be validated to establish differences in reported results and these differences be taken into account.

D. Inadequate specimen collection, transport, storage, and processing may lead to inaccurate results.

E. This assay has been validated for use with Ahlstrom/Munktel TFN and Whatman 903 DBS cards. Ensure that DBS cards are validated to meet lab-specific requirements.

F. Ensure DBS cards are handled and stored in accordance with manufacturer instructions.

Performance for DBS

Limit of Detection (LoD) Using the 3rd HIV-1 WHO International Standard

The limit of detection (LoD) is defined as the concentration of HIV-1 RNA that is detected at 95% or greater probability according to CLSI EP17-A2 (7). The LoD was determined by testing panels that consisted of dilutions of the 3rd HIV-1 WHO International Standard (subtype B, NIBSC code: 10/152) in HIV-1 negative whole blood. Thirty replicates of each dilution were run on three Panther systems using three reagent lots for a total of 90 replicates for each dilution. Per CLSI EP17-A2, the results from the reagent lot with the highest concentration for the predicted detection limit are defined as LoD and are shown in Table 3. Through Probit analysis, the LoD for the Aptima HIV-1 Quant Dx assay is 873.88 copies/mL (95% Confidence Interval 653.98–1,311 copies/mL) or 2496.8 IU/mL (95% Confidence Interval 1868.5–3745.72 IU/mL, 0.35 copies = 1 IU).

Table 3: LoD of the Aptima HIV-1 Quant Dx Assay With DBS Using the 3rd HIV-1 WHO International Standard

Predicted Detection Limit	Concentration (copies/mL)	Concentration (IU/mL)
10%	49.21	140.60
20%	75.61	216.01
30%	103.05	294.42
40%	134.26	383.60
50%	171.93	491.22
60%	220.17	629.04
70%	286.86	819.58
80%	390.97	1117.05
90%	600.69	1716.24
95%	873.88	2496.80

Linear Range

The linear range of the Aptima HIV-1 Quant Dx assay was established by testing panels that consisted of cultured HIV-1 subtype B virus diluted in HIV-1 negative whole blood according to CLSI EP06-A (8). Panels ranged in concentration from 2.70 to 7.60 log copies/mL. Testing was performed on four Panther systems with two reagent lots of Aptima HIV-1 Quant Dx assay. As shown in Figure 4, the Aptima Quant Dx assay demonstrated linearity across the range tested.

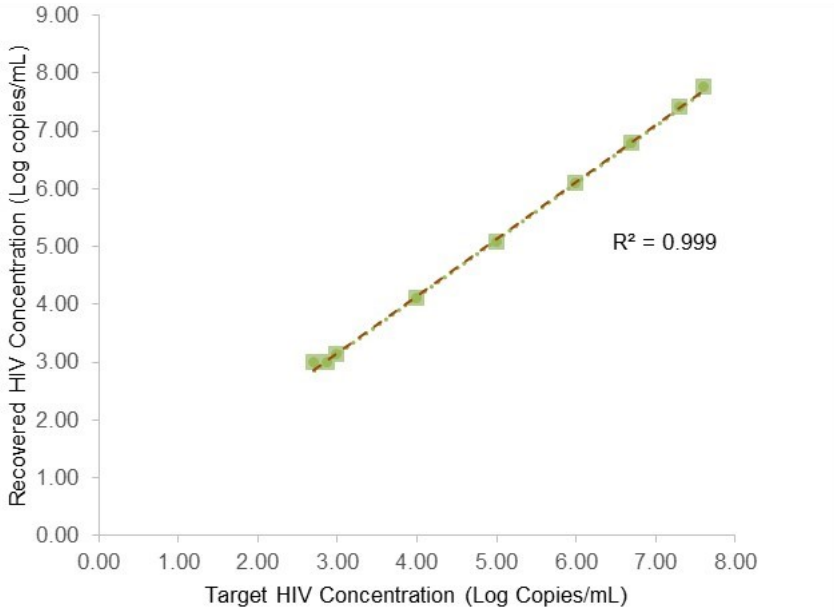

Figure 4. Linearity of the Aptima HIV-1 Quant Dx Assay With DBS

Lower Limit of Quantitation Using the 3rd HIV-1 WHO International Standard

The lower limit of quantitation (LLoQ) is 883 copies/mL. The LLoQ was established as described in CLSI EP-17-A2 to meet LLoQ requirements of >95% reactivity and a total error of ≤1 log c/mL.

Table 4: Determination of Lower Limit of Quantitation with DBS sample type using the 3rd HIV-1 WHO International Standard with 3 Reagent Lots

Reagent Lot	% Positive	Target Concentration (Log copies/mL)	Aptima HIV-1 Quant Dx (log copies/mL)	SD (log copies/mL)	| Bias | (log copies/mL)	Calculated Westgard TE (log copies/mL)
1	90%	2.78	2.61	0.60	0.17	1.37
	93%	2.85	2.66	0.54	0.18	1.26
	93%	2.90	2.83	0.40	0.07	0.87
	97%	2.95	**2.90**	0.18	0.06	0.43
	100%	3.00	2.98	0.22	0.02	0.46
	100%	3.08	3.03	0.22	0.05	0.48
2	93%	2.78	2.57	0.59	0.20	1.38
	97%	2.85	2.76	0.44	0.09	0.97
	93%	2.90	2.66	0.54	0.24	1.32
	97%	2.95	2.79	0.47	0.17	1.10
	97%	3.00	2.78	0.46	0.22	1.15
	97%	3.08	**2.91**	0.37	0.17	0.91
3	100%	2.78	2.80	0.52	0.02	1.05
	100%	2.85	2.83	0.36	0.01	0.74
	100%	2.90	2.89	0.36	0.02	0.74
	100%	2.95	2.94	0.50	0.02	1.01
	100%	3.00	**2.95**	0.23	0.05	0.51
	97%	3.08	3.07	0.16	0.01	0.33

Table 5: Summary of LLoQ

Reagent Lot	LLoQ (log copies/mL)	LLoQ (copies/mL)
1	2.90	787
2	2.91	817
3	2.95	883

Precision

To assess precision of the Aptima HIV-1 Quant Dx assay, a panel was made by spiking cultured HIV-1 subtype B virus into HIV-1 negative whole blood. Three operators using three reagents lots tested the panels on three Panther systems over 20 days (see Table 6). The panel consisted of one HIV-1 negative panel member and five HIV-1 positive panel members. Assignment of the concentration for clinical specimens or cultured virus stocks was determined by testing the DBS sample type in the Aptima HIV-1 Quant Dx assay.

Table 6: Precision of the Aptima HIV-1 Quant Dx Assay With DBS

Number of Valid Replicates	Mean Concentration (log copies/mL)	Inter-Instrument SD	Inter-Operator SD	Inter-Lot SD	Inter-Run SD	Intra-Run SD	Total SD
79	3.45	0.00	0.00	0.07	0.00	0.20	0.21
81	3.94	0.00	0.00	0.07	0.08	0.13	0.13
81	4.81	0.01	0.01	0.01	0.07	0.07	0.07
81	5.89	0.00	0.03	0.01	0.07	0.05	0.05
81	6.72	0.00	0.02	0.02	0.06	0.05	0.05

SD=standard deviation

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD=0. The total replicates tested for each panel member was 81; only replicates with quantifiable results were used to assess precision.

Potentially Interfering Substances

The susceptibility of the Aptima HIV-1 Quant Dx assay to interference by elevated levels of hemoglobin and human DNA was evaluated by testing DBS prepared from whole blood in the absence of HIV-1 and presence of 3.42 and 4.7 log copies/mL of HIV-1. No interference in performance was observed in the presence of Hemoglobin (5 mg/mL) and human genomic DNA (2 µg/mL).

The Aptima HIV-1 Quant Dx assay has also been evaluated for interference for plasma specimens and no interference in performance was observed in the presence of exogenous and endogenous substances. For the complete list of potentially interfering substances that were evaluated for the plasma sample type, refer to *Aptima HIV-1 Quant Dx Assay* package insert (AW-11853).

Specificity

Specificity of the Aptima HIV-1 Quant Dx assay was determined by testing DBS specimens prepared with blood from 500 HIV-1 negative donors across three reagent lots. The specificity of the assay with DBS was 99.6% (95% Confidence Interval 98.6% to 99.9%).

Analytical Specificity

Potential cross-reactivity to pathogens present in whole blood was evaluated in the Aptima HIV-1 Quant Dx assay by testing DBS prepared from whole blood spiked with 1e6 cells/mL of each organism in the absence of HIV-1 and presence of 3.42 and 4.7 log copies/mL of HIV-1. No interference in performance was observed in testing DBS containing *Leishmania major*, *Trypanosoma gambiense*, *Babesia microti Gray*, *Plasmodium falciparum*, and *Toxoplasma gondii* in the presence and absence of HIV-1.

The Aptima HIV-1 Quant Dx assay has also been evaluated for cross-reactivity for plasma specimens, and no interference in performance was observed in the presence of pathogens. For the complete list of pathogens that were evaluated for the plasma sample type, refer to *Aptima HIV-1 Quant Dx Assay* package insert (AW-11853).

Clinical Performance

Diagnostic Agreement for Early Infant Diagnosis

To assess diagnostic agreement, DBS specimens were prepared from heel stick or finger stick from infants ≤ 18 months of age born to HIV-1 positive mothers in Kenya, Africa. These infants were tested using a single DBS per test in the Aptima HIV-1 Quant Dx assay and a comparator CE-marked HIV-1 qualitative assay. As shown in Table 7, 1975 specimens had valid results in both assays. For the CE-marked qualitative comparator assay, all specimens with reactive results were retested and only confirmed reactive results were categorized as "Detected." All nonreactive sample results were categorized as "Target Not Detected." For Aptima,assay results interpreted as equivocal (see Table 2) were retested. The diagnostic agreement for early infant diagnosis between the two assays was 99.6%.

Table 7: Diagnostic Agreement Between Aptima HIV-1 Quant Dx Assay and Comparator Assay

		CE-Marked Comparator Assay	
		Target Not Detected	**Detected**
Aptima HIV-1 Quant Dx Assay	**Target Not Detected**	1888	4
	Detected	3	80

Method Correlation

The performance of the Aptima HIV-1 Quant Dx assay for the DBS sample type was assessed by comparing the DBS results to the Aptima assay plasma result. A total of 258 HIV-1 infected patients were enrolled in this study from 5 collection sites across Kenya, Africa. From each patient, DBS specimens were prepared using both capillary blood (finger stick) and venous blood. Plasma was also obtained from the same patient. All Aptima assay testing for DBS and plasma specimens was conducted with one lot of reagents. The results from specimens that were quantified with each sample type were analyzed by least squares linear regression as shown in Figures 5, 6, and 7.

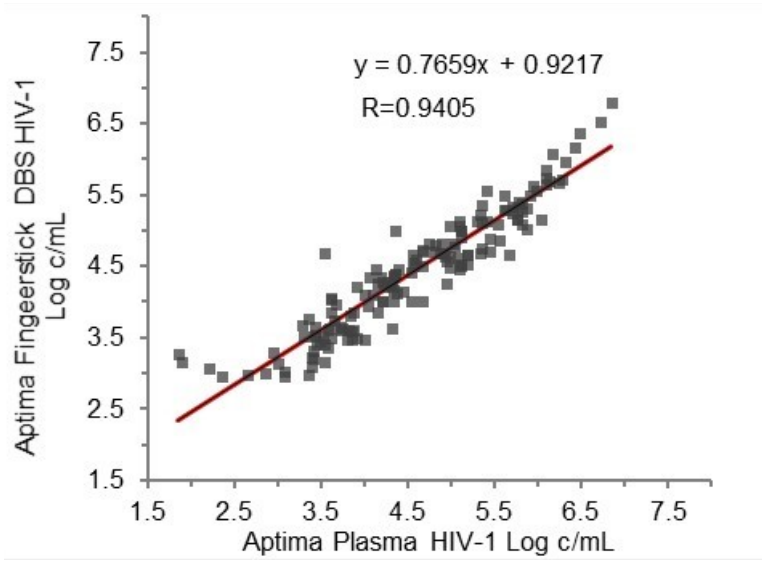

Figure 5. Correlation Between Finger Stick DBS and Plasma

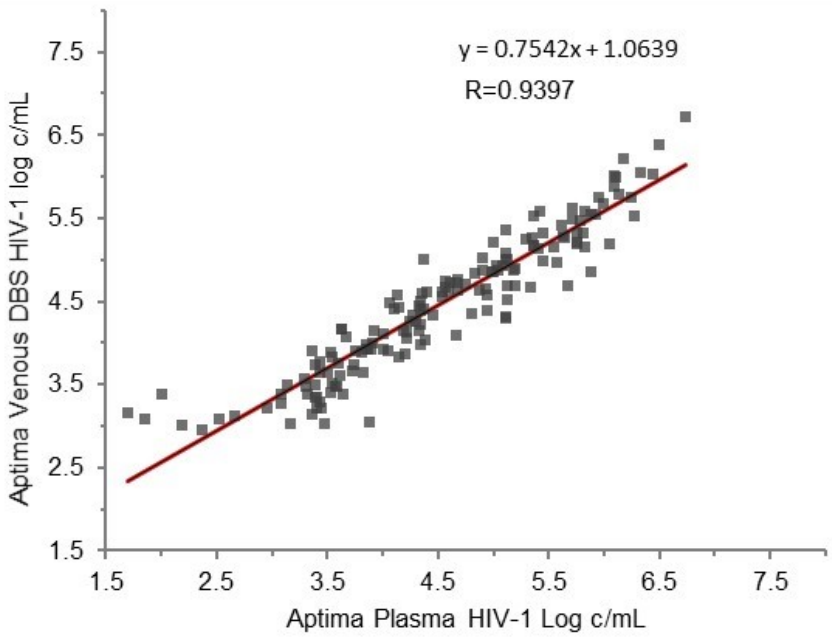

Figure 6. Correlation Between Venous DBS and Plasma

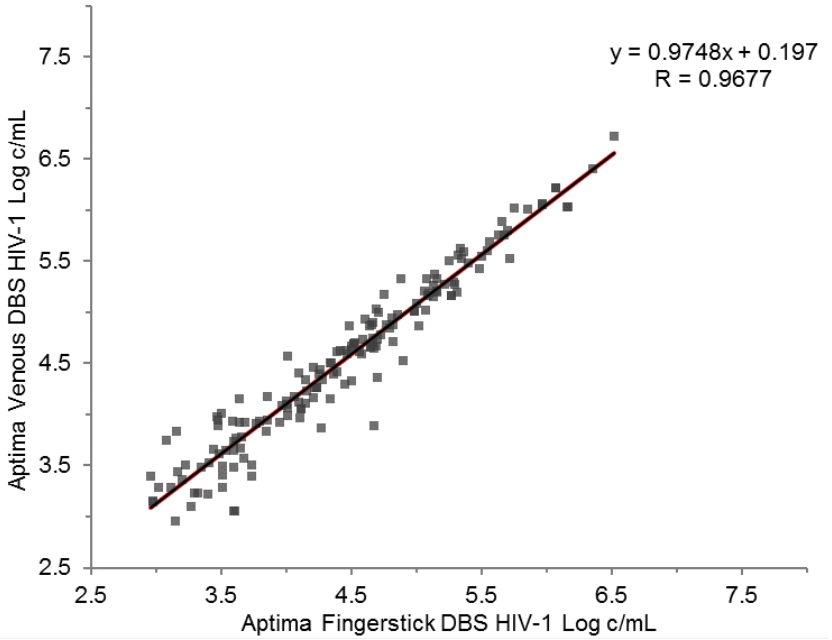

Figure 7. Correlation Between Finger Stick DBS and Venous DBS

The agreement of DBS and plasma results were also assessed at a threshold of 1000 c/mL. (Tables 8 and 9). The positive and negative agreement between finger stick DBS and plasma results were 92.95% and 93.14%, respectively. The positive and negative agreement between venous DBS and plasma results were 96.15% and 90.20%, respectively. The positive and negative agreement between venous DBS and fingerstick DBS results were 91.25% and 93.88%, respectively. The total agreement of HIV-1 results for plasma with the HIV-1 results from finger stick DBS and venous DBS were 93.02% and 93.80%, respectively.

Table 8: Agreement Between Finger Stick DBS and Plasma in the Aptima HIV-1 Quant Dx Assay

		Aptima Plasma	
		<1000	**>1000**
Aptima Finger Stick DBS	**<1000**	95	11
	>1000	7	145

Table 9: Agreement Between Venous DBS and Plasma in the Aptima HIV-1 Quant Dx Assay

		Aptima Plasma	
		<1000	**>1000**
Aptima Venous DBS	**<1000**	92	6
	>1000	10	150

Table 10: Agreement Between Venous DBS and Finger Stick DBS in the Aptima HIV-1 Quant Dx Assay

		Aptima Venous DBS	
		<1000	**>1000**
Aptima Finger stick DBS	**<1000**	92	**14**
	>1000	6	**146**

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AW-18108-001 Rev. 002
2020-07