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.(7) 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. High humidity may require longer drying time.
- 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.

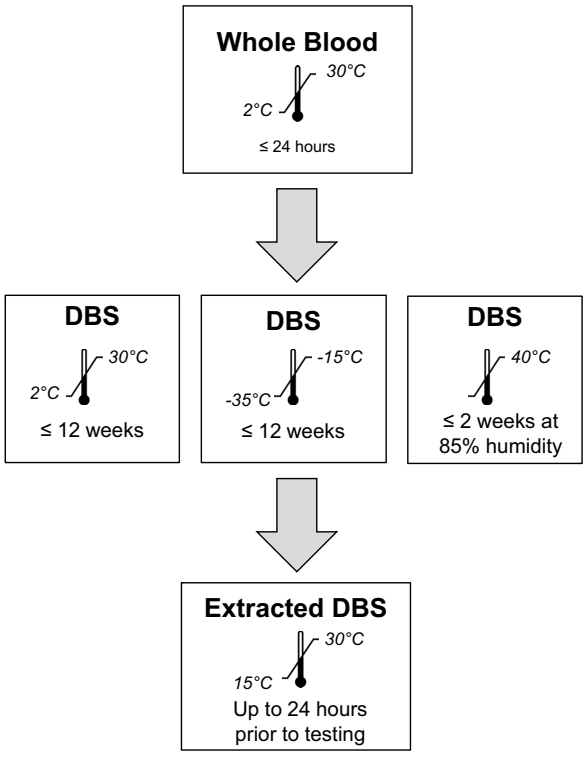

Figure 1. Storage Conditions for DBS

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	*CL0041 (100 caps)*
TCR bottle	*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 Panther, centrifuge SAT containing the extracted DBS for 2 minutes at 3,000 g.
7. Load SAT containing the DBS on Panther (extracted DBS may be stored on Panther 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.*

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.

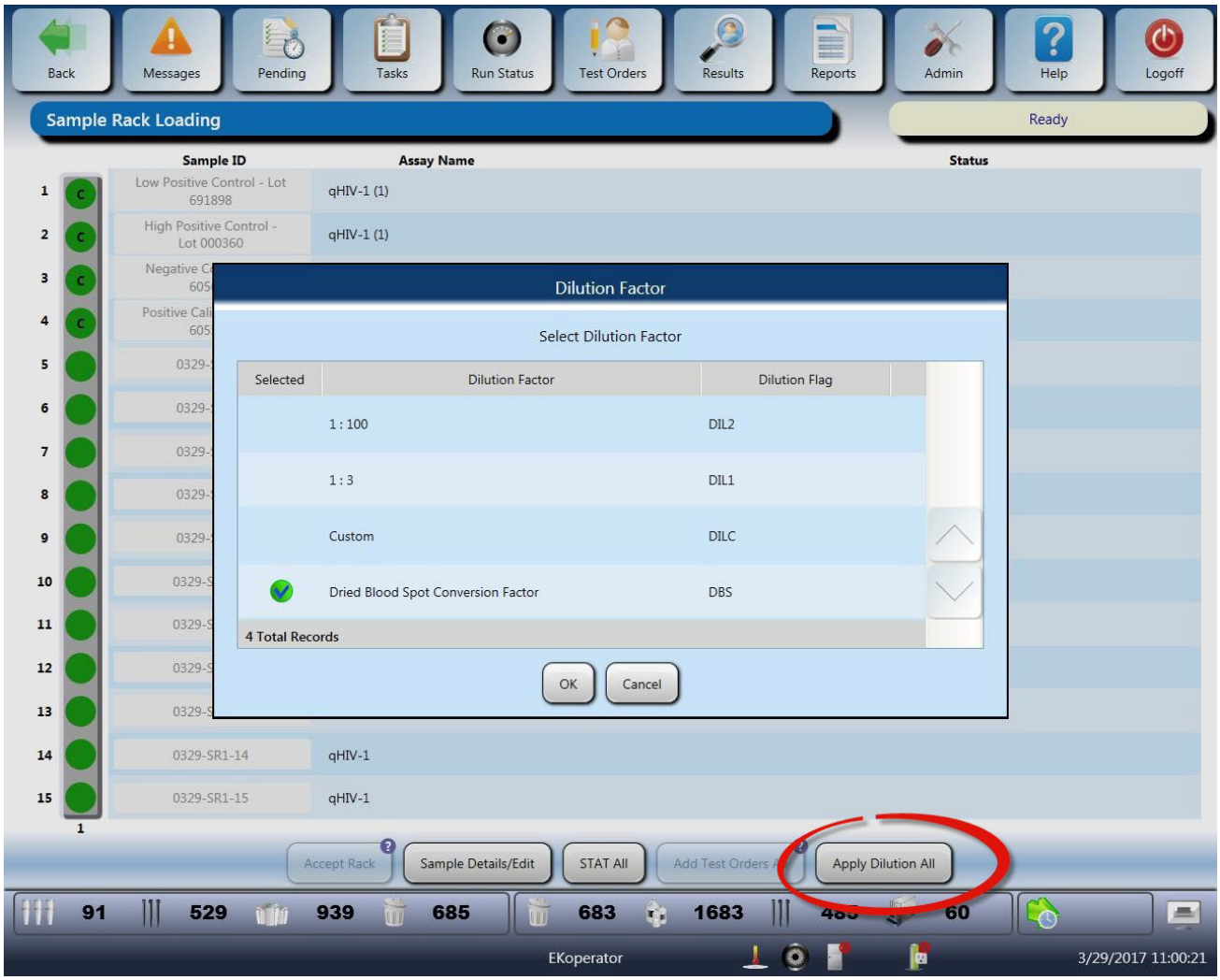

Figure 2. The Dilution Factor Window in the Sample Rack Loading Screen

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.

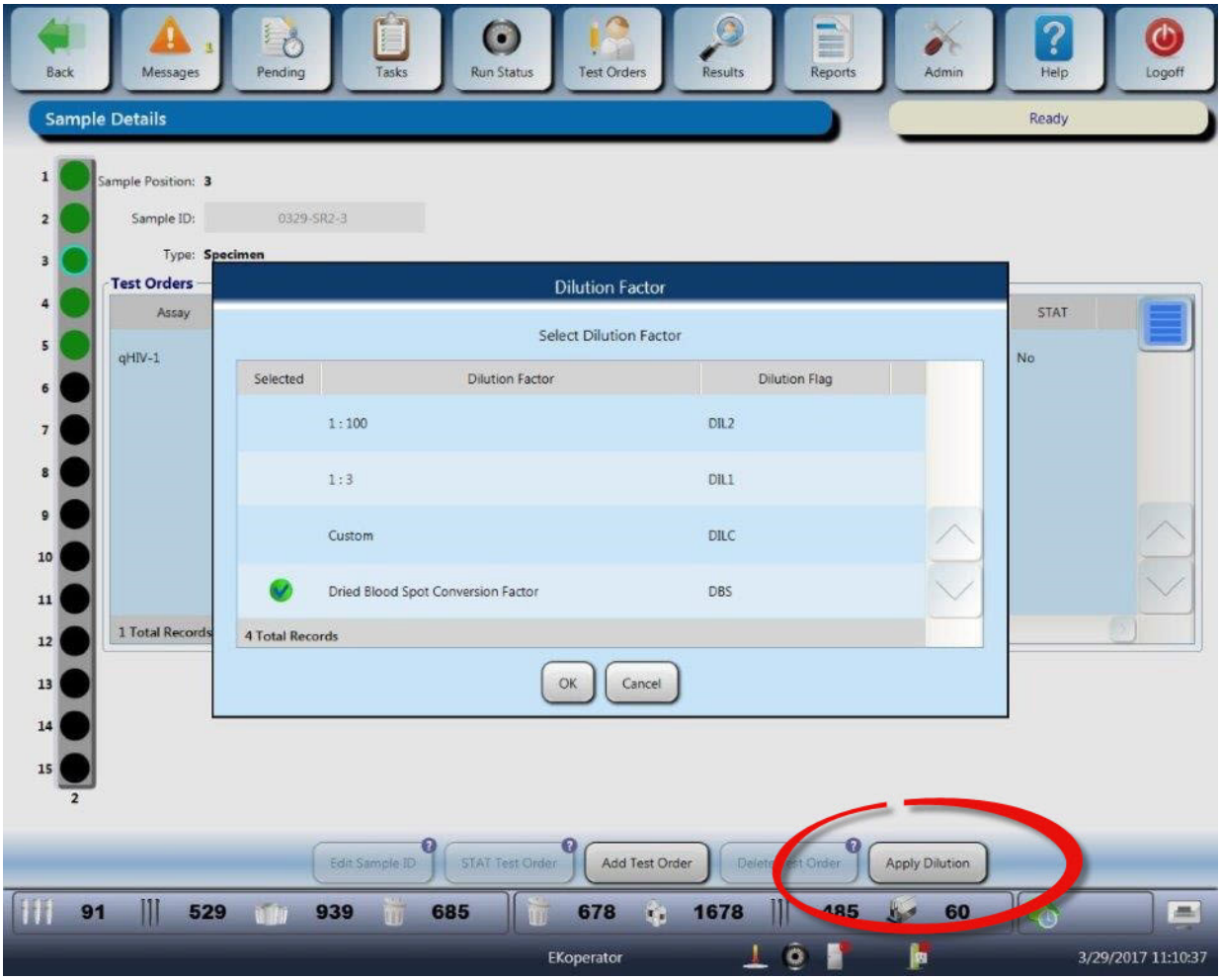

Figure 3. The Dilution Factor Window in the Sample Details Screen

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, deselect **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 of 29.1. The log conversion for DBS LoD of 873 c/mL is 2.94 log c/mL. The 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 1). Specimens with results listed as “Not Detected” are nonreactive for HIV-1 RNA. Specimens with results listed as “<873 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 DBS Specimens

Reported Aptima HIV-1 Quant Dx Assay Result		HIV-1 RNA Concentration Interpretation	User’s Diagnostic Qualitative Interpretation
Copies /mL	**$\log_{10}$ Value[a]**		
Not Detected	Not Detected	HIV-1 RNA not detected.	Non-reactive for HIV-1 RNA
<873 detected	<2.94	HIV-1 RNA is detected but at a level below the lower limit of quantitation of DBS (LLoQ DBS 873 copies/mL)	Retest to confirm reactive diagnostic results. Only confirmed positives are considered reactive.[b]
873 to 10,000,000	2.94 to 7.00	HIV-1 RNA concentration is within the linear range of DBS (873 copies/mL 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[c]	Invalid[c]	There was an error in the generation of the result. Specimen should be retested.	Invalid

[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 of HIV: HIV Treatment—Interim Guidance. Geneva, Switzerland. http://www.who.int/hiv.

[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 (8). 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 2. 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 2: 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 (9). 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.

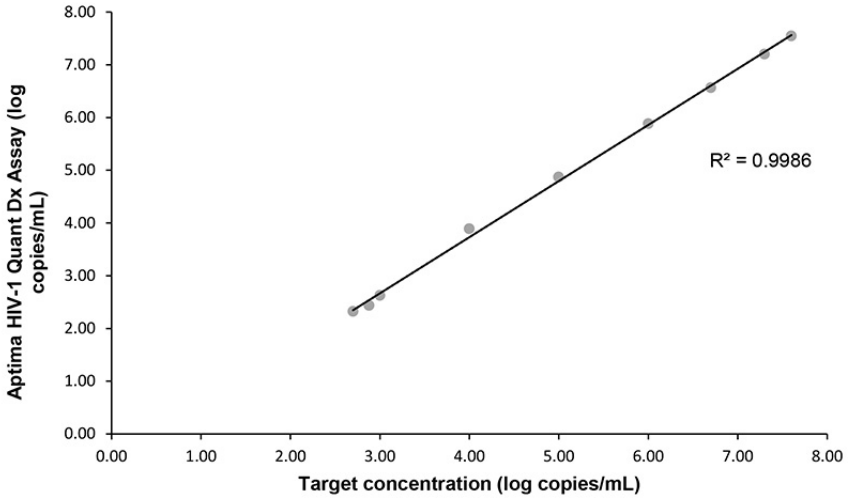

Figure 4. Linearity of the Aptima HIV-1 Quant Dx Assay With DBS

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

The lower limit of quantitation (LLoQ) is equal to LoD of 873.88 copies/mL (95% Confidence Interval 653.98 – 1311 copies/mL) or 2496.8 IU/mL (1868.5 – 3745.72 IU/mL). The LLoQ was confirmed as described in CLSI EP-17-A2 to meet LLoQ requirements of >95% reactivity and a total error of ≤1 log c/mL.

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 3). 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 3: Precision of the Aptima HIV-1 Quant Dx Assay With DBS

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 (%)
64	3.25	0.02	0.65	0.00	0.00	0.07	2.15	0.00	0.00	0.17	5.21	0.18	5.67
81	3.71	0.00	0.00	0.00	0.00	0.07	1.90	0.08	2.14	0.14	3.75	0.17	4.72
81	4.60	0.01	0.15	0.01	0.19	0.01	0.25	0.07	1.61	0.07	1.50	0.10	2.23
81	5.68	0.00	0.00	0.03	0.55	0.01	0.20	0.07	1.30	0.05	0.84	0.09	1.66
81	6.51	0.00	0.00	0.02	0.35	0.02	0.25	0.06	0.93	0.05	0.71	0.08	1.25

CV=coefficient of variation, 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 and CV=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 presence and absence of 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. The specificity of the assay with DBS was 99.2% (95% Confidence Interval 98.0% to 99.7%).

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 presence and absence of
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 4, 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." The diagnostic agreement for early infant diagnosis between the two assays was 99.6%.

Table 4: 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 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 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.

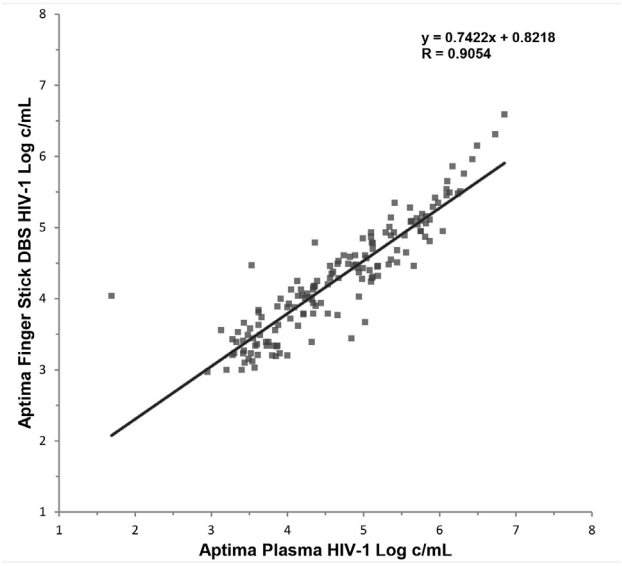

Figure 5. Correlation Between Finger Stick DBS and Plasma

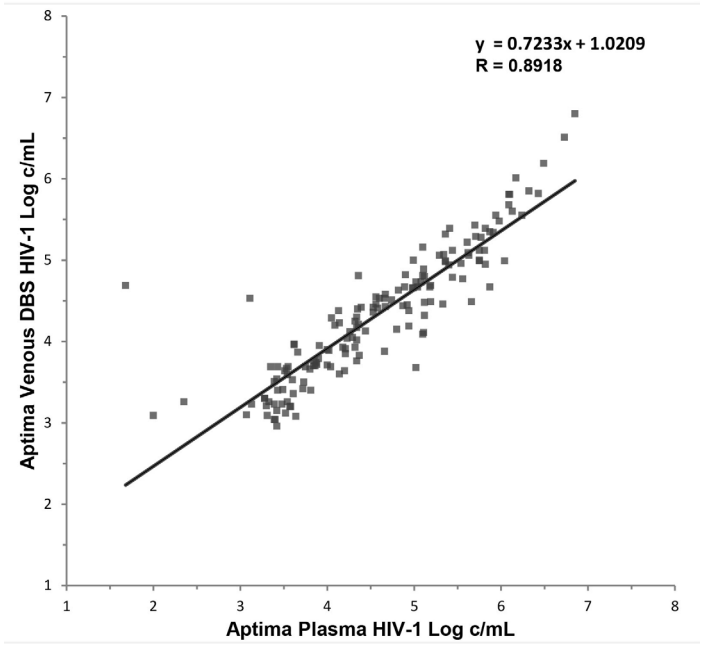

Figure 6. Correlation Between Venous DBS and Plasma

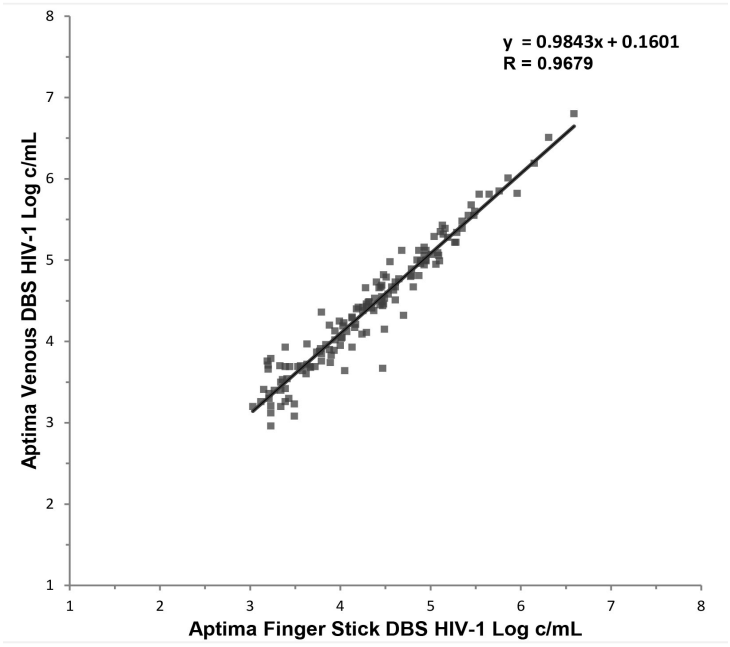

Figure 7. Correlation Between Finger Stick DBS and Venous DBS

The agreement of DBS and plasma results were also assessed at a threshold of 1000 c/mL. (Tables 5 and 6). The positive and negative agreement between finger stick DBS and plasma results were 89% and 97%, respectively. The positive and negative agreement between venous DBS and plasma results were 91% and 96%, respectively. The total agreement of HIV-1 results for plasma with the HIV-1 results from finger stick DBS and venous DBS were 92.2% and 93.0%, respectively.

Table 5: Agreement Between Finger Stick DBS and Plasma in the Aptima HIV-1 Quant Dx Assay

		Aptima Plasma	
		<1000	**>1000**
Aptima Finger Stick DBS	**<1000**	99	17
	>1000	3	139

Table 6: Agreement Between Venous DBS and Plasma in the Aptima HIV-1 Quant Dx Assay

		Aptima Plasma	
		<1000	**>1000**
Aptima Venous DBS	**<1000**	98	**14**
	>1000	4	**142**

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AW-17780-001 Rev. 003
2019-01