SUMMARY OF SAFETY AND EFFECTIVENESS (SSE)

I. GENERAL INFORMATION

Device Generic Name:	Not Applicable
Device Trade Name:	Aptima HIV-1 Quant assay
Product Code:	MZF
Applicant's Name and Address:	Hologic, Inc.
Manufacturer:	Hologic, Inc. 10210 Genetic Center Drive San Diego, CA 92121
Date of Panel Recommendation:	Not Applicable
Premarket Approval Application (PMA) Number:	BP150318
Office's Signatory Authority:	Jay S. Epstein, M.D. Director, OBRR/CBER

□ I concur with the summary review.
□ I concur with the summary review and include a separate review to add further analysis.
□ I do not concur with the summary review and include a separate review.

Date of Notice of Approval:	December 22, 2016
Expedited:	Not Applicable

Material Reviewed/Consulted: The PMA, amendments to the PMA, and other specific documentation used in developing the Summary of Safety and Effectiveness (SSE)

Review memos from the following reviewers were used in developing the SSE:

Discipline Reviewed	Reviewer Names
Preclinical Studies and Clinical Studies	Krishnakumar Devadas Uros Djekic* Andrew Dayton Pawan Jain Viswanath Ragupathy
Product Design	Krishnakumar Devadas Jiangqin Zhao
Chemistry/Manufacturing/Controls (CMC)	Krishnakumar Devadas Deborah Trout Jiangqin Zhao
Instrumentation and Software	Sajjad Syed
Statistician	Chunrong Cheng Paul Hshieh Tie-Hua Ng
Bioresearch Monitoring Inspection (BIMO)	Dennis Cato
DMPQ/pre-approval inspection	Deborah Trout
Labeling OCBQ/DCM/APLB	Krishnakumar Devadas Dana Jones
Policy	Pradip Akolkar Indira Hewlett J. Peyton Hobson David Leiby Hira Nakhasi Sayah Nedjar

* No longer with the Agency

II. INTENDED USE

The Aptima HIV-1 Quant assay is an *in vitro* nucleic acid amplification test (NAAT) for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals on the fully automated Panther system. The Aptima HIV-1 Quant assay quantitates HIV-1 RNA groups M, N, and O over the range of 30 to 10,000,000 copies/mL. One international unit is equivalent to 0.35 copies of HIV-1 RNA for the 3rd HIV-1 WHO International Standard (subtype B, NIBSC code: 10/152).

The Aptima HIV-1 Quant assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis, and for use as an aid in monitoring the effects of antiretroviral treatment, as measured by changes in plasma HIV-1 RNA levels.

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

CLIA COMPLEXITY: HIGH

III. DEVICE DESCRIPTION

The Aptima HIV-1 Quant assay is an *in vitro* nucleic acid amplification test (NAAT) for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals on the fully automated Panther system. The Aptima HIV-Quant assay is a real-time assay to quantitate HIV-1 RNA that uses real-time monitoring of Transcription Mediated Amplification (TMA). The Panther system provides full automation of all assay steps including sample processing, amplification of nucleic acid, detection and quantitation, and data analysis. The assay includes dual-target amplification and detection systems, targeting two regions of the HIV-1 genome (pol and LTR) independently.

Assay Principle

The main principles for the Aptima HIV-1 Quant assay involve three basic steps:

- Target capture
- Target amplification by a modified form of Transcription Mediated Amplification (TMA)
- Real time detection of amplicons using fluorescent labeled probes

The entire process takes place in a single tube. An internal calibrator / internal control (IC) in the assay serves as the control for nucleic acid capture, amplification and detection step errors, as well as the control for operator and instrument errors.

Assay Procedure

During target capture, viral nucleic acid is isolated from plasma specimens. Specimens are 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. 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 is achieved by a transcription mediated nucleic acid amplification (TMA) method that utilizes two enzymes, Moloney murine leukemia virus reverse transcriptase (MMLV-RT) 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 the RNA amplicon from the DNA copy template. The Aptima HIV-1 Quant assay utilizes the TMA method to amplify two regions of HIV-1 RNA (pol and LTR). Independent signals are generated from amplification of the pol and LTR regions using specific primers which are designed to amplify HIV-1 groups M, N, and O.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of each target 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 to 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 amplicons a stronger fluorescent signal is generated. The time taken for the fluorescent signal of each target to reach a specified threshold (T Time) is proportional to the starting HIV-1 RNA 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 calculated using the T Time for both pol and LTR amplification by the Panther system software. The software returns a single, clinically validated result based on an algorithm that utilizes the independent pol and LTR signals in comparison with stored calibration information.

Components of the Aptima HIV-1 Quant assay
The Aptima HIV-1 Quant assay kit (100 tests) for use with the automated Panther system consists of three reagent boxes:

Box 1: Aptima HIV-1 Quant assay kit which contains the following reagents:

- Amplification Reagent: The Amplification Reagent is a (b) (4) s, and ribonucleotides, when reconstituted allow for amplification of specific HIV-1 sequences and the HIV-1 Internal Control (IC) transcript. It contains raw materials necessary to build amplicons.
- Enzyme Reagent: The Enzyme Reagent is used in target amplification, via transcription mediated nucleic acid amplification (TMA). It contains two enzymes: recombinant Moloney murine leukemia virus reverse transcriptase (MMLV-RT) and recombinant bacteriophage T7 RNA polymerase (T7 polymerase) lyophilized in a HEPES buffered solution containing (b) (4)
- Promoter Reagent: The Promoter Reagent is a (b) (4) that when reconstituted allow for amplification and detection of specific HIV-1 sequences and the HIV-1 Internal Control (IC) transcript.
- Amplification Reconstitution Reagent: The Amplification Reconstitution Solution is a buffered solution containing a surfactant and glycerol used to reconstitute the lyophilized Amplification Reagent.
- Enzyme Reconstitution Reagent: The Enzyme Reconstitution Solution is a HEPES buffered solution containing a surfactant and glycerol used to reconstitute the lyophilized Enzyme Reagent.
- Promoter Reconstitution Reagent: The Promoter Reconstitution Solution is a buffered solution containing a surfactant and glycerol used to reconstitute the lyophilized Promoter Reagent.

- Target Capture Reagent: The Target Capture Reagent (TCR) is a HEPES-buffered suspension containing (b) (4) oligonucleotide, HIV and internal control (IC) specific oligonucleotides and an internal control transcript. The (b) (4) any virus that may be present in the donor sample and to inactivate nucleases. The capture oligonucleotides hybridize to the (b) (4) oligonucleotide on the magnetic particles and also hybridize with the target RNA during sample preparation. A magnetic field is applied to separate the target nucleic acid, mRNA attached to the magnetic particles, from the sample matrix.

Box 2: Aptima HIV-1 Quant Controls kit which contains the following reagents:

- Negative Control: The Negative Control contains HIV-1 negative defibrinated human plasma containing gentamicin and 0.02% sodium azide as preservatives.
- Low Positive Control: The Low Positive Control contains Non-infectious HIV-1 Armored RNA (2.60-3.30 $\log_{10}$ copies/ml) in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.
- High Positive Control: The High Positive Control contains Non-infectious HIV-1 Armored RNA (4.5 – 5.5 $\log_{10}$ copies/mL) in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.

Box 3: Aptima HIV-1 Quant Calibrator kit which contains the following reagent:

- Positive Calibrator: The Positive Calibrator contains HIV-1 transcript in a buffered solution at a concentration of approximately 500 copies/mL.

Additional Materials Required but Sold Separately
The Aptima Assay Fluids kit (also known as Universal Fluids Kit) which contains the following reagents:

- Wash Solution - (b) (4)
- Buffer for Deactivation Fluid - (b) (4)
- Oil Reagent - (b) (4)

Instrumentation and Software

The Panther Instrument – Overview
The Panther System is an integrated nucleic acid testing system that fully automates all steps necessary to perform Hologic assays from sample processing through amplification, detection, and data analysis.

The two main components of the Panther instrument are the analyzer and the computer workstation. The analyzer holds all of the fluids, reagents, and consumables needed to perform the assay. The analyzer contains an embedded Central Organization Processor (COP) module and additional control modules for all of the major sub-components of the analyzer. The COP and control modules contain the firmware responsible for controlling the analyzer. The computer workstation contains the software responsible for downloading the assay processing instructions to the COP, communicating system status and results. Users interact with the software through a touch-sensitive display and handheld barcode reader. An optional printer and

uninterruptible power supply (UPS) are accessories present outside of the instrument housing. The software in the computer workstation assembles a sequence of events that is transferred to the COP which then directs the distributed control modules to perform a set of operations. Each operation performed by a control module is monitored for correct operation by the COP and the overall status of the operation is returned to the software running on the computer workstation.

The Panther system detects and/or quantitates nucleic acid from specimens using Hologic assay technologies by automating the following assay processing steps:

1. Sample processing - Target Capture process isolates and purifies the target nucleic acid from clinical specimens.
2. Amplification and Detection – In this step, Transcription Mediated Amplification of target produces multiple copies of RNA that are detected in real-time using fluorescent probes. The fluorescence increases over time as nucleic acid complementary to the probe is generated.
3. Results Report Generation - Assay results from each sample are determined by measuring Relative Fluorescence Units (RFU) over time, calculating an emergence time and converting this time to concentration using linear regression.

IV. TEST PROCEDURE

Specimen Collection, Preparation and Storage

- The Aptima HIV-1 Quant assay can be performed on plasma samples collected in ethylenediamine tetra acetic acid (EDTA) or Acid Citrate Dextrose (ACD) anticoagulants and plasma preparation tubes (PPT).
- Whole blood may be stored and/or transported at 2°C to 30°C for up to 24 hours prior to plasma separation.
- Plasma can be tested on the Panther system in the primary tube or transferred to the secondary Aptima Specimen Aliquot Tube (SAT).
- Upon separation EDTA and ACD plasma samples may be stored in primary collection tubes at 30°C for up to 24 hours or at 2°C to 8°C for up to 3 days. If transferred to the SAT, plasma may be stored at 2°C to 8°C for up to 5 days or stored frozen for up to 90 days at -20°C to -70°C. Specimens in EDTA or ACD primary collection tubes should not be frozen.
- Plasma preparation tubes (PPT) containing centrifuged plasma may be stored at 30°C for up to 24 hours or for up to 3 days at 2°C to 8°C. If transferred to the SAT, plasma may be stored for up to 5 days at 2°C to 8°C. For long term storage plasma may be stored frozen at -20°C to -70°C for up to 90 days in PPT or SAT.
- Plasma samples are stable for up to three freeze-thaw cycles.
- HIV-1 viral load quantitation plasma specimens may be diluted in the SAT.
- Specimens must be tested immediately after dilution and should not be frozen.

Running the Aptima HIV-1 Quant assay

The minimum sample volume required for running the Aptima HIV-1Quant assay is 400 μL. The test procedure is described in detail in the Panther System Operator's Manual and Procedural Notes.

Procedural steps are summarized below:

- Set up the system according to the instructions in the Panther System Operator's Manual and Procedural Notes
- Prepare the calibrators and controls
- Reconstitute and prepare the Target Capture, Amplification, Enzyme and Promoter Reagents
- Load reagents and consumables as prompted by the system
- Load samples into the Sample Rack
- Load the HIV-1 positive calibrator, HIV-1 low positive control, HIV-1 high positive control, and HIV-1 negative control tubes into the Sample Rack in any position and in any Sample Bay Lane on the Panther system
- Once the racks are loaded with samples and controls the assay process starts
- After amplification and signal detection is complete, the Output Queue performs deactivation of each reaction by (b) (4) into each tube
- After deactivation, residual liquid is aspirated to the Liquid Waste Container and the tubes are disposed of in the solid waste container
- Assay results from each sample are determined and reported by the Panther system

Procedural Notes

1. Specimens can be tested with the reconstituted Aptima HIV-1 Quant assay kit and associated calibrator and controls for up to 24 hours unless:
    - The calibrator or control results are invalid.
    - The associated assay reagent kit is removed from the system.
    - The associated assay reagent kit has exceeded the stability limits.
2. The calibrator and each control tube can only be used once. Using the tube more than once can lead to processing errors.

V. RESULTS

The Aptima HIV-1 Quant assay was clinically validated using the algorithm in the Panther system software. The validated result to be reported is provided by the Panther system software in the Results screen and Results report. The Panther system automatically determines the concentration of HIV-1 RNA for specimens and controls by comparing the results to a calibration curve. Assay results can be reported in copies/mL or $\log_{10}$ copies/mL. The conversion factor for copies to International Unit (IU) traceable to the WHO 3rd International Standard for HIV-1 RNA (10/152) is stored in the Panther software. Specimens diluted 1:3 or 1:100 can be tested using the 1:3 or 1:100 option, respectively, on the Panther system. The software will automatically report the neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

Quality Control Procedures

The Aptima HIV-1 Quant assay contains three quality control procedures:

- **Assay Calibration:** The calibration curve used for quantitation of clinical samples is generated using two points. The first point required to generate a calibration curve is generated by Hologic for each reagent lot by running several calibrators distributed across the assay range on multiple Panther instruments. The mathematical equation for the calibration curve is established and the point at which the line would cross the x-axis

determined by extrapolation. This calibration coefficient information is supplied to the user in the form of a reagent lot-specific bar code which is sent to the user along with the reagent master kit. This calibration coefficient barcode is scanned into the Panther system and stored in the software. The second point required for generation of calibration curve is generated by the user by testing 3 replicates of a calibrator within the run. Before the user is able to generate results, each reagent kit must also be calibrated. This is done by the user running 3 replicates of a Hologic supplied calibrator. The calibrator is composed of an *in vitro* transcript of HIV-1 diluted in a buffered solution to a concentration of 500 copies/mL. Due to the linear nature of the calibration curve, the Panther software is able to generate a reagent kit specific calibration curve using a combination of the user-run calibrator and the calibration coefficient supplied by Hologic. This calibration curve is then stored in the Panther system software and is valid up to 24 hours.

- **Assay Controls:** In order to generate valid results from patient specimens the user must also test assay run controls at least every 24 hours, or if the reagents are removed from the Panther system and reloaded. Three control materials are provided by Hologic to the user. The two positive controls are composed of HIV-1 armored RNA diluted in human plasma at known concentrations (one low concentration of 2.9 $\log_{10}$ copies/ml and one high concentration of 5 $\log_{10}$ copies/ml). These controls must generate results within a predefined concentration range for the calibration curve to be valid. A Hologic provided negative control must also give non-reactive results for the run to be valid.

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

Assay Validity

The validity of each sample result and each Aptima Quant HIV-1 assay run are automatically determined by the assay software.

Sample Validity is determined from several factors:

- The internal control (IC) result must be valid for a sample result to be valid.
- Specification ranges for time of emergence of the amplification curve, slope of the amplification curve and relative fluorescence units (RFU) range signal of internal control (IC) are stored in the Panther software and samples that fail to meet these predefined specifications will be invalidated.

Run Validity is determined by the calibrator and control performance:

- A reagent kit must have at least two valid calibrator replicates to generate a calibration curve. Outlier analysis using the Grubbs test is performed for the 3 replicates of calibrator. At least 2 of the 3 replicates of Positive Calibrator must be valid with a precision of <0.25 standard deviation log copies and recovery within 0.3 logs of expected concentration for it to be used for generation of calibration. The calibrator signals must also meet the relative fluorescence units (RFU) range specification for both HIV-1 targets and internal control (IC).

- The negative control must give a valid result for internal control (IC) and a "not detected" result for both HIV-1 targets for the run to be valid.
- The positive controls must recover within the expected concentration range for both pol and LTR targets. They must also meet the relative fluorescence units (RFU) range specification for sufficient signal for both HIV-1 targets and internal control (IC).
- The calibrator and positive controls also have additional specifications for T slope and T time that must be met for the run to be valid. The time at which the normalized amplification curve for a sample rises above a threshold value is called time of emergence or T time. The ratio of the T time of the HIV-1 target region to the T time of the internal control in the same tube is called the ratio value. The slope of the regression line between low and high bound points of a normalized amplification curve created using the fluorescent data from the Panther Real Time Fluorometer (RTF) is called the T slope. Minimum T slope values of (b) (4) were set for pol and IC targets to be considered positive. A ratio specification of (b) (4) was established as a threshold for positivity for the pol target.

VI. INTERPRETATION OF RESULTS

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 results generated by the Panther System can be interpreted as outlined in Table 1. The Aptima HIV-1 Quant assay has dual-target amplification and detection systems, targeting pol and LTR independently. The result automatically reported by the system will be based on the primary target, pol, unless pol is not amplified. In this case, the system will report the result from the secondary target, LTR. After results are available in the Results screen, the quantitation value for each target can be accessed using the Sample Curve Report feature in the Panther system software. The values and amplification curves can be viewed by selecting the Sample ID for the sample in the Results screen of the Panther system software and selecting the "Curve Data" button. A window will open containing the Sample Curve Report, which includes fluorescence profiles and quantitation values for the sample. Data obtained from the Sample Curve Report are provided for information only. The validated result to be reported for the sample is provided by the Panther system software in the Results screen and Results report.

Table 1: Results Reporting

Reported Aptima HIV-1 Quant Assay Result		**HIV-1 RNA Concentration Interpretation**
Copies/mL*	**$\log_{10}$ Value**	
Not Detected	Not Detected	HIV-1 RNA not detected
<30 detected	<1.47	HIV-1 RNA is detected but at a level below Lower Limit of Quantification (LLOQ)
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
>10,000,000	>7.00	HIV-1 RNA concentration is above Upper Limit of Quantitation (ULOQ)

Reported Aptima HIV-1 Quant Assay Result		HIV-1 RNA Concentration Interpretation
Copies/mL*	Log_{10} Value	
Invalid**	Invalid**	There was an error in the generation of the result. Specimen should be retested

*The conversion factor for copies to International Unit (IU) for the 3rd WHO International Standard for HIV-1 RNA (10/152) is 0.35 copies/IU.
**Invalid results are displayed in blue-colored font.

The results reporting algorithm for this assay was set up so that any sample that is reactive for either of the 2 HIV-1 targets would be assigned a "detected" result. Only those samples that have a non-reactive result for both HIV-1 targets are assigned a "target not detected" result. Data from a total of 7,028 HIV-1 negatives samples tested using 7 reagent lots was analyzed prior to finalization of assay cutoffs.

VII. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- The Aptima HIV-1 Quant assay is only intended for quantitation of HIV-1 viral load and is not intended for initial clinical diagnosis of HIV-1 infection.
- The Aptima HIV-1 Quant assay is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.
- 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

- 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.
- Only personnel adequately trained in the use of the Aptima HIV-1 Quant assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.
- Use only supplied or specified disposable laboratory ware.
- Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powder free gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- 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.
- Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations. Thoroughly clean and disinfect all work surfaces.
- 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.

- Good standard practices for molecular laboratories should include environmental monitoring.

Specimen Related

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

- Quantitative results of the Aptima HIV-1 Quant assay have been evaluated with plasma. Use of this test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.
- Do not use the reagent kit, the calibrator, or the controls after the expiration date.
- 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.
- Avoid microbial and nuclease contamination of reagents.
- Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents.
- Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

Procedural Limitations

- The use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in the package insert may result in erroneous results.
- Reliable results are dependent on adequate sample collection, transport, storage, and processing.
- Though rare, mutations within the highly conserved regions of a viral genome covered by the primers and/or probes in the Aptima HIV-1 quant assay may result in under-quantitation of or failure to detect the virus.

VIII. CONTRAINDICATIONS

There are no known contraindications for use for this test.

IX. ALTERNATIVE PRACTICES AND PROCEDURES

There are other FDA approved alternative devices for the *in vitro* quantitation of HIV-1. These assays, including the Aptima HIV-1 Quant assay, provide a means of measuring baseline HIV-1 level and monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

X. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The possibilities of erroneous results may occur due to device malfunction or operator error. An erroneously high test result may indicate therapeutic failure and/or a higher likelihood of progression to AIDS. A false result may also result in unnecessary treatment and/or psychological trauma to a patient. An erroneously low test result may lead to lack of appropriate treatment and/or instill a false sense of security in a patient which could lead to worsening of the patient's condition. The risks of erroneous test results are inherent in all *in vitro* diagnostic products. However, if appropriate directions are followed as stated in the package insert, the likelihood of erroneous results from the use of this device are minimal.

XI. MARKETING HISTORY

The Aptima HIV-1 Quant assay was CE-marked on November 6, 2014. The Aptima HIV-1 Quant assay and Aptima Specimen Diluent Kit are currently marketed in countries outside of the United States.

The Panther system is currently marketed for other Aptima assays in the United States and other countries.

The Aptima HIV-1 Quant assay, Aptima Specimen Diluent kit, and the Panther system have not been withdrawn from the market for reasons related to safety or effectiveness.

XII. SUMMARY OF PRE-CLINICAL STUDIES

Analytical Sensitivity

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

LoD was determined by testing panels that consisted of dilutions of the 3rd HIV-1 WHO International Standard (the National Institute for Biological Standards and Control (NIBSC) code: 10/152) in HIV-1 negative plasma. A total of 13 panel members (0, 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 250, and 750 IU/mL) were tested. Thirty replicates of each dilution were run on three Panther systems using three reagent lots for a total of 90 replicates for each dilution. Through Probit analysis, the LoD (95%) for the Aptima HIV-1 Quant assay was determined as 12 copies/mL (35 IU/mL; 0.35 copies = 1 IU). The results are shown in Table 2.

Table 2: HIV-1 RNA Limit of Detection (LoD)

HIV-1 RNA IU /mL	HIV-1 RNA copies /mL	Predicted Detection Limit
3.3	1.2	10%
4.6	1.6	20%
5.7	2.0	30%
7.2	2.5	40%
8.8	3.1	50%

HIV-1 RNA IU /mL	HIV-1 RNA copies /mL	Predicted Detection Limit
11	3.8	60%
14	4.8	70%
18	6.2	80%
26	9.0	90%
35	12.1	95%

Summary: The LoD study as designed demonstrated that the Aptima HIV-1 Quant assay detected HIV-1 RNA at a concentration of 12 (95% CI: 9.7 – 16.7) copies/mL or 35 (95% CI: 27.7 – 47.9) IU/mL. The 0.35 copies/IU conversion factor used in this assay is traceable to the 3rd HIV-1 International WHO Standard (NIBSC Code 10/152).

Limit of Detection for HIV-1 Group M subtypes, Group O and Group N

The panels used to determine the LoD for each subtype or group were made by spiking HIV-1 negative human plasma with clinical specimens (subtype A, CRF01_AE, CRF02_AG, C, D, F, and G) or isolates (Group O and N). Each panel consisted of six panel members at the following concentrations: 2, 5, 10, 15, 30 and 40 copies/mL. Each panel member was tested in 30 replicates with two reagent lots for a total of 60 replicates per panel member. Probit analysis was performed to generate 50% and 95% predicted detection limits.

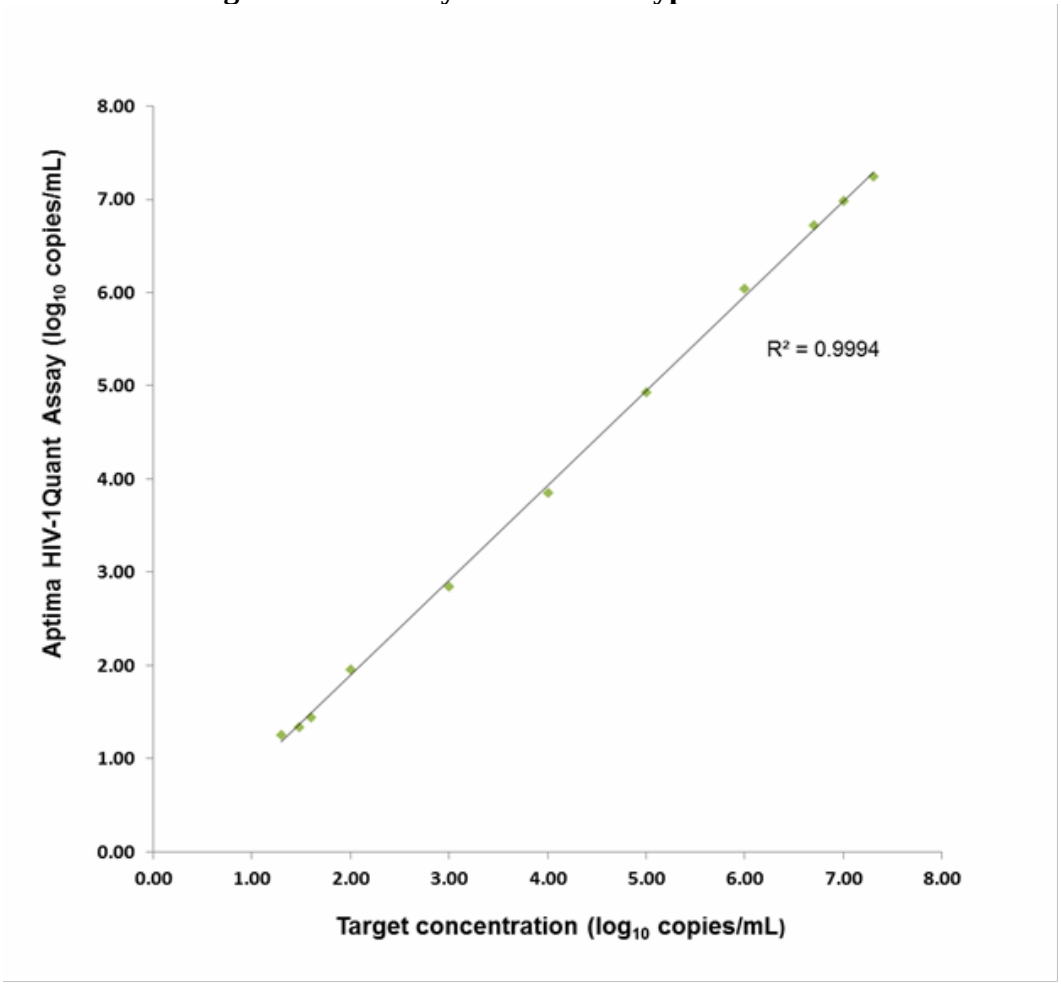
Table 3: Summary of Probit Analysis Results in copies /mL for HIV-1 Group M subtypes, Group N and Group O

Subtype / Group	Predicted Detection Limit	Concentration HIV-1 RNA 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

Summary: The results (Table 3) from this study demonstrated that the Aptima HIV-1 Quant assay has a LoD (95%) of 12.3 copies/mL with subtype A, 6.2 copies/mL with subtype CRF01_AE, 15.4 copies/mL with subtype CRF01_AG, 10.7 copies/mL with subtype C, 14.0 copies/mL with subtype D, 8.3 copies/mL with subtype F, 17.5 copies/mL with subtype G, 7.8 copies/mL with group N, and 8.0 copies/mL with group O.

Linearity of HIV-1 subtype B virus: The linear range of the Aptima HIV-1 Quant assay was established by testing panels that consisted of cultured HIV-1 subtype B virus diluted in HIV-1 negative human plasma (Figure 1). Panels ranged in concentration from 1.30 to 7.30 $\log_{10}$ copies/mL.

Figure 1: Linearity of HIV-1 subtype B virus



Summary: The Aptima HIV-1 Quant assay on the Panther system displayed a linear response from 20 copies/mL to 5 x 10^7 copies/mL (1.30 $\log_{10}$ copies/mL to 7.30 $\log_{10}$ copies/mL) with an R2 of 0.9994 on testing with 2 reagent lots. The non-linearity value (difference in recovery between the linear model and best fit model) according to the linearity analysis in CLSI EP06-A was 0.07 $\log_{10}$ copies/mL or less among all concentrations tested. The performance of the Aptima HIV-1 Quant assay is acceptable.

Linearity of HIV-1 Group M (subtypes A-H), Group N, and Group O prepared with HIV-1 Transcript stock: The linear response of the Aptima HIV-1 Quant assay for Group M subtypes A, B, C, D, F, G, H, CRF01_AE, Group N and Group O was confirmed by testing panels that

consisted of HIV-1 transcript diluted in buffer at concentrations ranging from 2.00 to 6.70 $\log_{10}$ copies/mL.

Summary: The Aptima HIV-1 Quant assay displayed a linear response from 100 copies/mL to 5 x 10^6 copies/mL (2.00 $\log_{10}$ copies/mL to 6.70 $\log_{10}$ copies/mL) for all HIV-1 Groups and subtypes tested that were prepared from a HIV-1 transcript stock. HIV-1 Group M Subtypes C, AE, G, H, and Group N were statistically linear according CLSI EP06-A linearity analysis. For HIV-1 subtypes A, B, D, F, and Group O the non-linearity value was 0.05 $\log_{10}$ copies/mL or less among all concentrations tested. The accuracy of quantitation for all subtype panels was within ±0.50 $\log_{10}$ copies/mL at 4.00 $\log_{10}$ copies/mL (1 x 10^4 copies/mL) for Groups M (subtypes A-H), N and O. The performance of the Aptima HIV-1 Quant assay is acceptable.

Linearity of HIV-1 Group M (subtypes A-H), Group N, and Group O prepared with clinical specimens or cultured virus: Dilutions of clinical specimens or cultured virus belonging to various subtypes were also tested. Clinical sample panels were targeted from 100 copies/mL to 1 x 10^5 copies/mL (2.00 to 5.00 $\log_{10}$ copies/mL). Panels of cultured HIV-1 virus were targeted from 100 copies/mL to 5 x 10^6 copies/mL (2.00 to 6.70 $\log_{10}$ copies/mL).

Summary: The Aptima HIV-1 Quant assay on the Panther system displayed a linear response from 100 copies/mL to 1 x 10^5 copies/mL (2.00 $\log_{10}$ copies/mL to 5.00 $\log_{10}$ copies/mL) for HIV-1 Group M (subtypes A1, B, C, CRF02_AG, and F1) clinical samples and a linear response from 100 copies/mL to 5 x 10^6 copies/mL (2.00 $\log_{10}$ copies/ to 6.7 $\log_{10}$ copies/mL) for cultured HIV-1 Group M (Subtype AE, G), Group N, and Group O samples. The clinical samples of HIV-1 Group M subtypes A1, B, C, CRF02_AG, F1 and AE were statistically linear according to CLSI EP06-A linearity analysis. For cultured HIV-1 Group M Subtype G, Group N, and Group O, the non-linearity value was 0.04 $\log_{10}$ copies/mL or less among all concentrations tested. The performance of the Aptima HIV-1 Quant assay is acceptable.

Lower Limit of Quantitation (LLoQ) using the 3rd HIV-1 WHO International Standard
LLoQ was determined by testing panels that consisted of dilutions of the 3rd HIV-1 WHO International Standard (NIBSC code: 10/152) in HIV-1 negative human plasma. The HIV-1 WHO standard is composed of HIV Group M, subtype B. A total of 6 panels were tested with three reagent lots in replicates of 30 for each lot from 23 runs.

Summary: The highest LLoQ across the three lots tested on the Aptima HIV-1 Quant assay using the 3rd HIV-1WHO International Standard was determined to be 15 copies/mL (1.17 $\log_{10}$ copies/mL). The performance of the Aptima HIV-1 Quant assay is acceptable.

Verification of LLoQ across HIV-1 subtypes and Groups
The LLoQ across HIV-1 subtypes and groups was verified. 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 cultured virus isolated from clinical samples. Testing consisted of a total 30 replicates per panel member.

Summary: The highest LLoQ for all subtypes and groups tested was determined to be 30 copies/mL (Table 4). The performance of the Aptima HIV-1 Quant assay is acceptable across the Group M subtypes, Group O and Group N samples evaluated in the study.

Table 4: Verification of LLoQ across HIV-1 subtypes and Groups

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 – Within Laboratory

The precision of the Aptima HIV-1 Quant assay on the Panther system was evaluated by testing panels that consisted of cultured HIV-1 subtype B virus diluted in HIV-1 negative human plasma (0 to 5 million copies/mL). The panel consisted of one HIV-1 negative panel member and eight HIV-1 positive panel members. Three operators tested 3 replicates of each sample in 2 runs for each of the 3 reagent lots on each of the 3 Panther systems. Testing was performed over 20 days. Each sample was tested neat and one sample was tested neat as well as diluted 1:3.

Table 5: Within Laboratory Precision of Aptima HIV-1 Quant assay

Number of valid replicates*	Mean Con. Log_{10} 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[a]	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[b]	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[c]	2.47[#]	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[d]	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

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 n tested for each panel was 162. Only replicates with numerical values were analyzed

[#] Panel member was diluted 1:3 just prior to run. Reported results (shown here) has dilution factor of 3

[a] Twenty-five replicates not included (1 replicate was invalid and 24 replicates were detected but not quantifiable)

[b] Five replicates not included (4 replicates were invalid and 1 replicate was detected but not quantifiable)

[c] Two replicates not included (2 replicates were detected but not quantifiable)

[d] Three replicates not included (3 replicates were invalid)

Summary: The total variability, expressed in log_{10} copies/ml was less than 0.17 log_{10} copies/mL for all samples (Table 5). The low variability seen on testing multiple concentrations with different Panther systems, reagent lots, and operators demonstrated that the assay has acceptable precision.

Performance with HIV-1 Negative Specimens

Specificity of the Aptima HIV-1 Quant assay was determined using 120 fresh and 510 frozen HIV-1 negative plasma specimens collected in tubes containing K3 EDTA (ethylenediamine tetra

acetic acid) as the anticoagulant. Testing was conducted at Hologic, Inc., using 3 reagent lots. HIV-1 RNA was not detected in all 630 samples (specificity of 100%; 95% CI: 99.4-100%).

Summary: The results obtained from testing of 630 HIV-1 negative plasma specimens using the Aptima HIV-1 Quant assay demonstrated acceptable performance since the test did not yield any false positive results.

Potentially Interfering Microbial Contaminants

Potential cross-reactivity and/or interference of pathogens in the Aptima HIV-1 Quant assay was evaluated in the presence or absence of cultured HIV-1 subtype B virus at a concentration of 3 $\log_{10}$ copies/mL in HIV-1 negative human plasma. Negative processed plasma was spiked with one of the following cultured pathogens at the target concentrations outlined in Table 6 below and tested on the Panther system using one reagent lot.

Table 6: Pathogens Tested for Cross-Reactivity

Pathogen	Final Target Concentration
Hepatitis A virus	100,000 PFU/mL[1]
Hepatitis B virus	100,000 IU/mL[2]
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[3]
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[4]
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[5]
Candida albicans	1,000,000 CFU/mL

[1]PFU/mL = Plaque forming units per mL
[2]IU/mL = International units per mL
[3]vp/mL = Viral particles per mL
[4]CFU/mL = Colony forming units per mL
[5]IFU/mL = Inclusion forming units per mL

Summary: The results demonstrated that there was no interference to the Aptima HIV-1 Quant assay performance in presence of spiked microbes in HIV-1 target negative or HIV-1 target positive specimens. The results demonstrate acceptable performance and indicate that the

presence of non-specific pathogens do not affect the analytical specificity and sensitivity of the Aptima HIV-1 Quant assay.

Potentially Interfering Substances (Endogenous)
The interference of elevated levels of endogenous substances (hemoglobin, bilirubin, triglycerides and protein), autoimmune disorder markers (antinuclear antibody, Rheumatoid Factor, and systemic lupus erythematosus), and markers of other diseases (Multiple Myeloma, Hyperglobulinemia, antibodies to HIV-2, HCV, and HBV) on the Aptima HIV-1 Quant assay was evaluated. HIV-1 negative plasma samples and samples spiked with cultured HIV-1 virus to a concentration of 3 $\log_{10}$ copies/mL (1000 copies/mL) were tested. Specificity was evaluated by testing the same set of analytical samples and clinical specimens in the absence of HIV-1 target in the Aptima HIV-1Quant assay. Testing was performed on 2 Panther systems using one reagent lot.

Summary: No interference to the performance of the Aptima HIV-1 Quant 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 was observed on testing clinical specimens that were lipemic, icteric or hemolyzed or those from patients with rheumatoid factor (RF), antinuclear antibody (ANA), anti-Jo-1 antibody (JO-1), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), hyperglobulinemia (IgG or IgM), elevated alanine aminotransferase (ALT), alcoholic cirrhosis (AC), multiple myeloma (MM), elevated protein albumin, HIV-2 antibodies, HCV antibodies, and HBV antibodies. The presence of these substances did not produce a detectable result in the absence of HIV-1 target.

Potentially Interfering Substances (Exogenous)
The susceptibility of the Aptima HIV-1 Quant assay to interference by drugs commonly prescribed to HIV-1 infected individuals was evaluated using HIV-1 negative plasma and samples spiked with cultured HIV-1 virus at a concentration of 3 $\log_{10}$ copies/mL. A total of 55 potentially interfering substances were divided into 12 pools and dissolved in their appropriate solvents at concentrations at least 20 times their intended test concentrations and tested on 2 Panther systems using one reagent lot (Table 7).

Table 7: Exogenous Substance Panels

Pool #	Exogenous Substances Tested	Pool #	Exogenous Substances Tested
Pool 1	lopinavir	**Pool 5**	paroxetine HCl
	indinavir		fluoxetine
	saquinavir		sertraline
	ritonavir	**Pool 6**	ganciclovir
	nelfinavir mesylate		valacyclovir
	darunavir		acyclovir
	amprenavir		rifampin/rifampicin
	atazanavir		ethambutol
Pool 2	nevirapine	**Pool 7**	ciprofloxacin
	efavirenz		azithromycin
	rilpivirine		amoxicillin
	clarithromycin		cephalexin
	amphotericin B		ampicillin

Pool #	Exogenous Substances Tested	Pool #	Exogenous Substances Tested
Pool 3	tenofovir disoproxil fumarate		trimethoprim
	adefovir dipivoxil	**Pool 8**	valganciclovir hydrochloride
	ribavirin		boceprevir
	enfuvirtide		telaprevir
	maraviroc		simeprevir
	raltegravir		sofosbuvir
	dolutegravir	**Pool 9**	pegylated interferon alpha -2b
Pool 4	abacavir sulfate		interferon alpha -2a
	didanosine		interferon alpha -2b
	zidovudine	**Pool 10**	heparin
	lamivudine		EDTA
	stavudine		sodium citrate
	entecavir	**Pool 11**	tipranavir
	telbivudine	**Pool 12**	isoniazid
	emtricitabine		

Summary: No interference in performance of the Aptima HIV-1 Quant assay was observed in the presence of the exogenous substances tested at concentrations at least three times the Cmax in human plasma.

Carryover

Carryover was evaluated as the rate of false positive results obtained with the Aptima HIV-1 Quant assay when samples containing high titer levels of HIV-1 were interspersed throughout specimen processing racks containing negative samples. HIV positive panel members were prepared by spiking cultured virus, (HIV-1 subtype B) into negative processed plasma at a target concentration of 6.89 to > 7.00 $\log_{10}$ copies/mL. Each run consisted of 42 HIV positive specimens and 52 negative processed plasma specimens. Five high-titer runs were performed on 2 Panther systems using one reagent lot.

Summary: The results of this study on the Panther system demonstrate that the presence of high titer HIV specimens interspersed throughout specimen processing racks containing negative samples does not cause false positive reactions when tested with the Aptima HIV-1 Quant assay. Carryover contamination risk in the presence of high titer specimens on the Panther system in this study was 0%.

Establishment of Traceability to the WHO Standard

This study was carried out to determine the conversion factor between Aptima HIV-1 Quant assay results in RNA copies/mL and WHO HIV-1 International Units/mL (IU/mL). The 3rd lot of HIV-1 WHO standard (NIBSC code 10/152) was used to standardize the Aptima HIV-1 Quant assay. To perform this study, 3 vials of the WHO standard were reconstituted to a concentration of (b) (4) The 3 vials of WHO standards (b) (4) These panels were tested in the Aptima HIV-1 Quant assay on the Panther system. A total of (b) (4) replicates were tested for each panel member (b) (4) replicates per reagent lot of reagents). To calculate the conversion factor between the Panther output copies/mL and WHO IU/mL, the Panther system result was (b) (4) WHO IU/mL to provide the copies/IU conversion factor for each panel member.

Summary: The results from this study demonstrated that the Aptima HIV-1 Quant assay has a 0.35 copies/IU conversion factor between HIV-1 RNA copies/mL and HIV-1 International Units/mL (IU/mL).

Reproducibility Study

Reproducibility of the Aptima HIV-1 Quant assay was evaluated on the Panther system at 3 external sites. Two operators performed testing at each site. Each operator performed 2 runs per day over 3 days, using 3 reagent lots over the course of testing. One Panther system was used to perform testing at each site. Each run had 3 replicates of each panel member. Reproducibility was tested using panel members (A, B, C, E, F and G) that consisted of HIV-1 spiked plasma. Positive panel members were created by spiking the negative plasma with cultured virus (HIV-1 subtype B) in concentrations that spanned the linear range of the Aptima HIV-1 Quant assay.

Table 8: Reproducibility

Panel Member	N	Mean Log_{10} Copies /mL	Between Sites (CV)	Between Operators (CV)	Between Lots (CV)	Between Days (CV)	Between Runs (CV)	Within Runs (CV)	Total (CV)
A	108	5.710	1.257	0.000	0.220	0.854	0.765	1.234	2.113
B	108	3.840	1.753	0.000	0.000	0.952	0.978	1.617	2.748
C	108	2.940	2.738	0.000	0.000	1.488	0.000	3.691	4.831
E	108	4.939	1.523	0.000	0.568	0.502	1.012	1.179	2.304
F	108	6.711	0.804	0.000	0.321	0.428	1.030	0.848	1.647
G	85[a]	1.844	3.513	0.000	0.967	0.000	3.073	8.982	10.169

CV=coefficient of variation

[a]Number of valid results with detectable HIV-1 RNA

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, CV is shown as 0.

Summary: Table 8 shows the reproducibility and precision of assay results for each positive panel member between sites, between operators, between lots, between days, between runs, within runs, and overall. When only samples with results above the LLoQ were included, total standard deviation (SD) was $\leq$0.2 log_{10} copies/mL for all panel members. When all samples with detectable HIV-1 RNA were included, total SD values remained unchanged except for panel member G, which had a total SD of 0.3 log_{10} copies/mL. For the HIV-1 negative panel member, 108 replicates were tested and HIV-1 RNA was not detected in all 108 replicates (negative agreement=100%, 95% Score CI: 96.6% to 100%).

Specimen Collection

Whole blood from 60 donors was collected in 4 different types of primary collection tubes; plasma preparation tubes (PPT), ethylenediamine tetra acetic acid（K2 EDTA and K3 EDTA) tubes and acid citrate dextrose (ACD) tubes. The samples were then spiked with HIV-1 subtype B cultured virus at a concentration of 4.00 log_{10} copies/mL (10,000 copies/mL), and tested.

Summary: The accuracy of quantitation for spiked positive plasma was within ± 0.50 $\log_{10}$ copies/mL of the expected value for plasma samples collected in PPT, K2 EDTA tubes, K3 EDTA tubes and ACD tubes.

Sample Handling (Dilution)
To assess sample dilution, a panel consisting of 11 samples with concentrations that spanned the linear range of the Aptima HIV-1 Quant assay was tested. The panel also contained two samples above the Upper Limit of Quantitation of the assay that were tested neat and diluted (1:3 or 1:100 in specimen diluent) in triplicate.

Summary: The results demonstrated that the Aptima HIV-1 Quant assay accurately quantitates samples diluted with Specimen Diluent. The standard deviations of results for the 10 clinical specimens tested three times were less than 0.25 $\log_{10}$ copies/mL (0.01 to 0.08 $\log_{10}$ copies/mL) demonstrating that results are reproducible.

Specimen Inhibition, Whole System Failure Rate
Evaluation of the whole system failure rate leading to false negative results was tested using low positive specimens. HIV positive panel members were prepared by spiking cultured HIV-1 subtype B virus, into negative human serum or negative plasma (n=179) to a targeted concentration of 100 copies/mL and tested. In addition, a total of 4 unspiked samples each for serum and plasma were also tested. Panels were tested with one reagent lot using 2 Panther systems. One run was performed on each of the instruments. There was one invalid reaction due to Operator error (Sample Quantity Not Sufficient) for a HIV-1 spiked sample.

Summary: All HIV-1 spiked serum and plasma samples were positive and there were no false negatives during this study. All unspiked samples tested were negative. There were no errors due to internal control failure and no false negatives due to whole system failure during testing. No chemistry invalid reactions (a failure of the Internal Control to amplify) were observed out of a total of 187 reactions. The results of this study demonstrated that the whole system failure rate (for both invalids and false negatives) was 0%, excluding instrument or operator error.

Sample Stability, Freeze Thaw
The Aptima HIV-1 Quant assay was evaluated with plasma samples tested as fresh and as frozen and thawed 3 times at -20°C and at -70°C to determine if quantitative performance is maintained. HIV-1 negative panel members, HIV-1 positive panel members consisting of HIV-1 negative specimens spiked with HIV-1 clinical specimens to a final concentration of 300 copies/mL and HIV-1 positive panel members consisting of HIV-1 negative specimens spiked with cultured HIV-1 virus to a final concentration of 1,000,000 copies/mL were tested.

Summary: Results from the spiked fresh and frozen plasma panel members demonstrated that unspiked HIV-1 negative plasma panel members were HIV-1 negative for all test conditions and all HIV-1 spiked panel members were HIV-1 positive for all test conditions. For panel members spiked with 300 copies/mL, the average copies/mL at fresh, -20°C freeze/thaw and -70°C freeze/thaw were 338, 316 and 380 copies/ml, respectively. For panel members spiked with 1,000,000 copies/mL, the average copies/mL at fresh, -20°C freeze/thaw and -70°C freeze/thaw were 1,148,153 copies/mL for all three conditions. The results from this study demonstrate acceptable quantitative performance of the Aptima HIV-1 Quant assay for samples frozen and thawed three times at -20°C and at -70°C.

Comparison of Fresh/Frozen Samples
Paired fresh and frozen HIV-1 positive clinical plasma samples were tested in duplicate with the Aptima HIV-1 Quant assay. Testing was performed in-house using 3 reagent kit lots. For samples with quantifiable HIV-1 RNA levels from both fresh and frozen testing (N=64), the averaged Aptima HIV-1 Quant assay results for each fresh sample were compared to the averaged results for the frozen sample.

Summary: The mean difference between fresh and frozen results was 0.06 $\log_{10}$ copies/mL. The median difference was 0.03 $\log_{10}$ copies/mL and the standard deviation was 0.175. The results demonstrated acceptable performance.

Real-Time Reagent Stability
Shelf life stability / Expiration dating of the Aptima HIV-1 Quant assay kit reagents (storage temperature: 2°C to 8°C) and the HIV-1 Quant assay Calibrators and Controls (storage temperature: -35°C to -15°C) were evaluated using 3 kit lots at the specified storage conditions. Real-time stability studies were evaluated from date of manufacture of kit lots. Open-kit stability was assessed at each stability time-point for the reconstituted Aptima HIV-1 Quant assay kit reagents after storage for an additional 38 days at 2°C to 8°C. On board stability was assessed for the reconstituted Aptima HIV-1 Quant assay kit reagents after storage for 90 hours (+ up to 4 hours) loaded uncapped on the Panther instrument. During these 90 hours, the reconstituted Aptima HIV-1 Quant assay kit was loaded on and off the Panther instrument to 2°C to 8°C storage for 5 cycles. The Aptima HIV-1 Quant assay Calibrator and Controls are single-use reagents and were tested at real-time time points only.

Summary: Results demonstrate acceptable performance for up to 17 months for the Aptima Quant HIV-1 assay reagents stored at 2°C to 8°C, with 30 days storage at 2°C to 8°C after opening the kit (reconstitution). The reagents can be stored on-board the Panther instrument for 72 hours. Results for Aptima HIV-1 Calibrator Kit demonstrate acceptable results for up to 18 months and the results for the Aptima HIV-1 Controls Kit demonstrate acceptable results for up to 17 months at -35°C to -15°C.

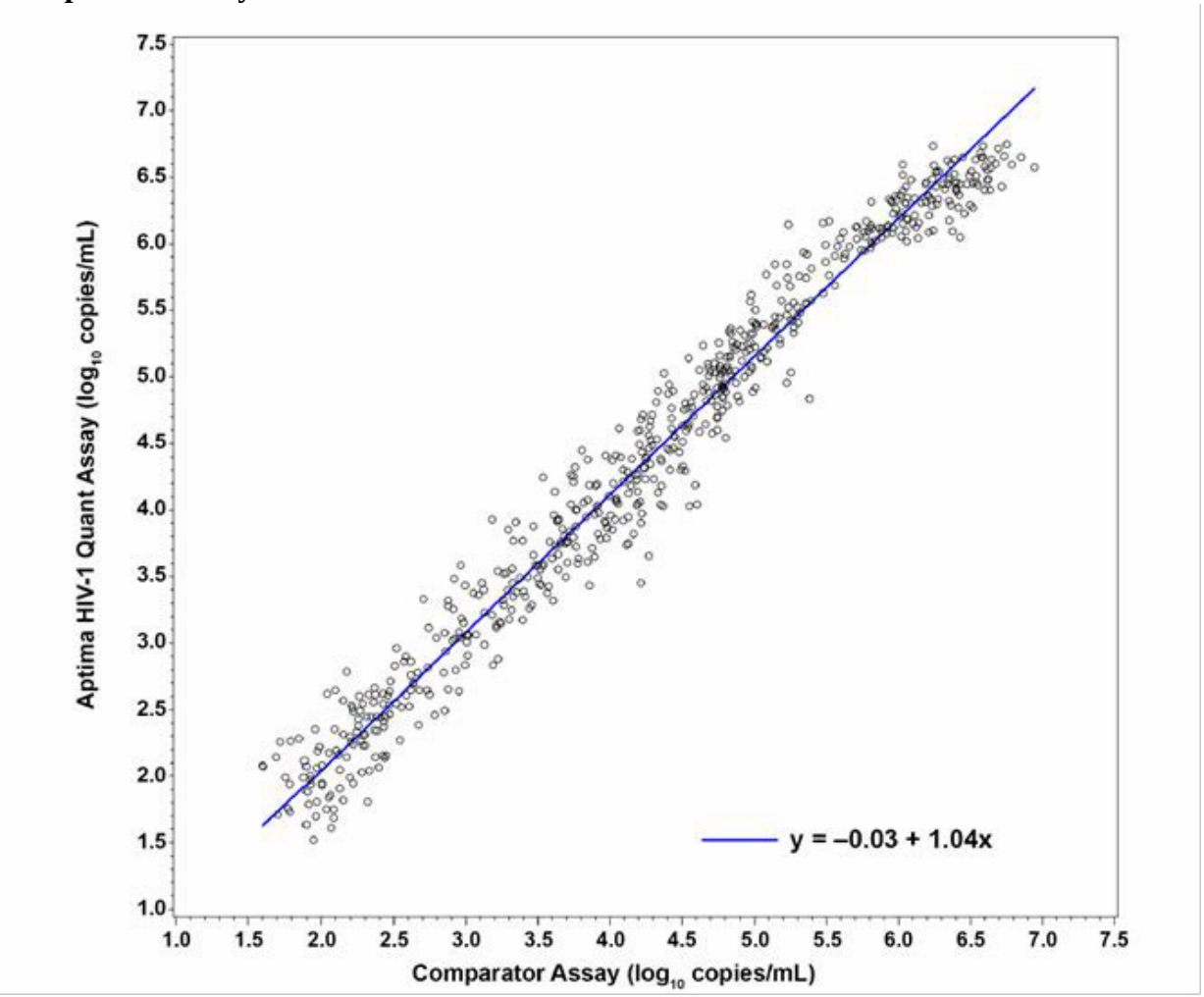
XIII. SUMMARY OF CLINICAL STUDIES

Validation of Viral Load Quantitation (Method Comparison Study)
Quantitation of HIV-1 RNA was compared between the Aptima HIV-1 Quant assay and an FDA approved viral load assay. The study included testing of clinical plasma samples (stored fresh or frozen) and contrived samples (cultured virus spiked into negative clinical plasma samples). Each sample was tested in duplicate with the Aptima HIV-1 Quant assay and the comparator assay. Aptima HIV-1 Quant assay testing was performed at 3 external sites, with each site using 3 reagent kit lots; comparator assay testing was performed at 1 external laboratory.

Summary: Results from 628 samples (within the linear range of both assays) were analyzed using Deming regression. Of these samples, 82 were clinical samples stored fresh (never frozen) before testing with the Aptima HIV-1 Quant assay and comparator assay. Figure 2 shows the scatter plot with Deming fit of the paired Aptima HIV-1 Quant assay and paired FDA approved viral load assay results for all testing sites. Overall, the constant bias (intercept) was -0.03 (95% CI: -0.10 to 0.03); the standard error was 0.033. The proportional bias (slope) was 1.04 (95% CI: 1.02 to 1.05); the standard error was 0.007 and the correlation coefficient was 0.99. The results

meet the statistical acceptance criteria, as the 95% CI for the intercept is within the range of >-0.5 to <0.5, the 95% CI for the slope is within the range of >0.9 to <1.1, and the correlation coefficient is ≥0.85.

Figure 2: Deming Regression Analysis between the Aptima HIV-1 Quant Assay and Comparator Assay



Viral Load Discrimination at Clinically Meaningful Thresholds

In the method study comparing the Aptima HIV-1 Quant assay with the comparator assay for 628 samples, concordance analysis was evaluated at the medical decision points of 50 and 200 copies/mL.

Table 9: Viral Load Discrimination at the Clinically Meaningful Threshold of 50 and 200 copies/mL

Decision Level (Copies/mL)	Site	Bias (Copies/mL)	95% CI (Copies/mL)		Range of Aptima HIV Quant Assay Result Based on 95% CI for the Bias (Copies/mL)	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound
50	All	1.07	0.97	1.18	50.97	51.18
200	All	1.13	1.04	1.22	201.04	201.22

CI = confidence interval

[1] Deming regression is performed under the assumption of constant standard deviation for both assays.

Summary: Based on the biases of the Aptima HIV-1 Quant assay in relation to the comparator, the expected Aptima results at the medical decision points of 50 and 200 copies/mL are 51.07 and 201.13 copies/mL, respectively (Table 9). Based on upper and lower bound of 95% CI for biases of the Aptima HIV-1 Quant assay in relation to the comparator, the expected range of Aptima HIV-1 Quant assay results at the medical decision point of 50 copies/mL, are 50.97 (50 + lower bound of 0.97) and 51.18 (50 + upper bound of 1.18) copies/mL. Based on upper and lower bound of 95% CI for biases of the Aptima HIV-1 Quant assay in relation to the comparator, the expected Aptima HIV-1 Quant assay results at the medical decision point of 200 copies/mL, are 201.04 (200 + lower bound of 1.04) and 201.22 (200 + upper bound of 1.22) copies/mL. The performance of the Aptima HIV-1 Quant assay to determine viral load across the medical decision points at 50 and 200 copies/mL is acceptable.

Clinical Specificity Study

To assess specificity, previously frozen HIV-1 negative plasma samples obtained from volunteer whole blood donors were tested with the Aptima HIV-1 Quant assay. Testing was performed at 3 external sites with 3 reagent kit lots. Clinical specificity was calculated as the percentage of HIV-1 negative samples with results of "Not Detected." Six hundred (600) HIV-1 negative plasma samples were tested.

Summary: Results demonstrated that HIV-1 RNA was not detected in all 600 samples. Specificity was determined to be 100% (600/600, 95% CI was 99.4% to 100%).

XIV. INSPECTIONS

Manufacturing Facilities Review/Inspection

Facility information and data provided in the PMA were reviewed by CBER and found to be sufficient and acceptable. The facilities involved in the manufacture of Aptima HIV-1 Quant assay and the Panther System are listed in the table below. The activities performed and inspectional histories are noted in the table below.

Manufacturing Facilities Table for Aptima HIV-1 Quant assay and Panther System

Name/address	FEI number	Inspection/ waiver	Results/ Justification
Final Device Manufacturer Hologic, Inc. Willow Court Facility 10808 Willow Ct San Diego, CA 92127 Hologic, Inc. Genetic Center Drive Facility 10210 Genetic Center Drive San Diego, CA 92121	2032600 2024800	Waived Waived	Team Biologics Level 2 inspection for both sites January 2016 NAI
(b) (4) (b) (4)	(b) (4)	(b) (4)	(b) (4) NAI

NAI – No Action Indicated

Bioresearch Monitoring (BIMO) Inspections

CBER BIMO issued inspection assignments to three sites in United States. These inspections did not reveal any deviations that impacted the data submitted in this PMA. The inspections were classified as No Action Indicated (NAI).

XV. CONCLUSIONS DRAWN FROM THE PRECLINICAL AND CLINICAL STUDIES

Safety Conclusions

Based on the results of the analytical and clinical studies, the Aptima HIV-1 Quant assay, when used according to the directions provided, should be safe and pose minimal risk to patients..

Effectiveness Conclusions

The clinical study results, in combination with the non-clinical performance evaluations including validation of viral load quantitation, strongly support the effectiveness of the Aptima HIV-1 Quant assay for the medical intended use to assess patient prognosis by measuring the baseline HIV-1 level and to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

Benefit-Risk Conclusions

The benefits outweigh the risks at the level of performance observed in the pivotal clinical study. Complimentary analytical studies strengthen this conclusion. Accurate quantitation of HIV-1 RNA is an essential component of the treatment of HIV-1 infection. In an era of highly active antiretroviral therapy for HIV infection, accurate quantitation of viral load to monitor

treatment and assess sustained virological suppression has substantial individual benefit (i.e., reduction of the risk of disease progression). Risk related to inaccurate quantitation was substantially mitigated by device design (i.e., use of controls) and Panther system process controls that include both hardware and software components (i.e., verification that the assay processing steps are correct for each reaction; verification of reaction incubation times and temperatures; and verification that reagents and fluids are appropriately dispensed). Appropriate warnings to address routine risks encountered in the laboratory practice are contained in the labeling and package inserts for the device. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the intended use. The data from the analytical studies demonstrated acceptable performance of the Aptima HIV-1 Quant assay when used according to the instructions for use as stated in the package insert. The clinical studies performed with the Aptima HIV-1 Quant assay and statistical analysis support the use of this device to quantitate HIV-1 RNA and the test can be used to measure both baseline and changes in HIV-1 RNA levels during the course of antiretroviral treatment.

XVI. PANEL RECOMMENDATIONS

Not Applicable – This product was not submitted for review by the Blood Products Advisory Committee.

XVII. FDA/CBER DECISION

The PMA BP150318 is recommended for approval.