DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

Hologic, Inc.
Ron Domingo
Regulatory Affairs Manager
10210 Genetic Center Dr.
San Diego, California 92121

June 15, 2017

Re: K162673
Trade/Device Name: Aptima Herpes Simplex Viruses 1 & 2 Assay
Regulation Number: 21 CFR 866.3305
Regulation Name: Herpes simplex virus serological assays
Regulatory Class: Class II
Product Code: OQO
Dated: May 16, 2017
Received: May 17, 2017

Dear Mr. Domingo:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements

as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and Part 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely,

Stephen J. Lovell -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K162673

Device Name
Aptima Herpes Simplex Viruses 1 & 2 Assay

Indications for Use *(Describe)*

The Aptima Herpes Simplex Viruses 1 & 2 assay (Aptima HSV 1 & 2 assay) is an *in vitro* diagnostic nucleic acid amplification test (NAAT), using real time transcription-mediated amplification (TMA), for the qualitative detection and differentiation of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) messenger RNA (mRNA) in clinician-collected swab specimens from anogenital skin lesions. The assay is intended for use with swab specimens placed in Aptima specimen transport medium (STM) or in viral transport media (VTM) that is immediately diluted into STM.

The Aptima HSV 1 & 2 assay is intended for use as an aid in the diagnosis of HSV-1 and/or HSV-2 infections in symptomatic male and female patients. The Aptima HSV 1 & 2 assay is indicated for use on the Panther® system.

Warning
The Aptima HSV 1 & 2 assay is not FDA-cleared for use with cerebrospinal fluid (CSF) or for prenatal screening.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

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510(k) SUMMARY
Aptima Herpes Simplex Viruses 1 & 2 Assay

I. SUBMITTER

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Date Prepared: June 14, 2017

II. DEVICE

Proprietary Name of Device:	Aptima Herpes Simplex Viruses 1 & 2 Assay
Common or Usual Name:	Nucleic acid amplification test for Herpes Simplex viruses
Classification Name:	Herpes simplex virus serological assays
Regulation Number:	21 CFR 866.3305
Regulatory Class:	Class II
Product Code:	OQO

III. PREDICATE DEVICE

The predicate device is the BD ProbeTec Herpes Simplex Viruses (HSV 1 & 2) Q^x Amplified DNA Assays (K103798; cleared March 18, 2011; BD Diagnostics Systems, Sparks, MD).

IV. DEVICE DESCRIPTION

The Aptima Herpes Simplex Virus 1 & 2 assay (Aptima HSV assay) is a nucleic acid amplification test (NAAT) developed for use on the fully automated Panther system that utilizes target capture, transcription mediated amplification (TMA), and real-time detection of HSV-1, HSV-2, and an internal control (IC). The Aptima HSV assay amplifies and detects mRNAs for

HSV-1 and HSV-2. These RNAs are expressed from the viral genome during the infection cycle, and are packaged inside HSV-1 and HSV-2 viral particles prior to virus release from infected cells. The Aptima HSV assay therefore detects virus-infected cells and the mature virus particles themselves.

The Aptima HSV assay involves three main steps, which all take place in a single tube on the Panther® system: target capture, target amplification by TMA, and detection of the amplification products (amplicon) by the fluorescent labeled probes (torches). The assay incorporates an IC in every test to monitor targeted nucleic acid capture, amplification and detection.

When the Aptima HSV assay is performed, the targeted viral mRNA and IC are isolated using magnetic microparticles and target-specific capture oligomers, in a process called target capture. The capture oligomers contain sequences complementary to specific regions of the targeted RNA (HSV mRNA or IC) as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the RNA target molecules. The microparticles, including the captured RNA target molecules bound to them, are pulled to the side of the reaction tube using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors. After target capture steps are completed, the specimens are ready for amplification.

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV (Moloney murine leukemia virus) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and hybridize specifically to the amplicon in real time. Each torch has a fluorophore and a quencher. The quencher suppresses the fluorescence of the fluorophore as it is designed to be in close proximity when not hybridized to the amplicon. 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. More torch hybridizes when more

amplicon is present. The increase in fluorescent signal from progressive amplification is detected by fluorometers within the Panther system. The Panther system can detect and discriminate between the three fluorescent signals corresponding to HSV-1, HSV-2 and IC amplification products. The fluorescence (measured in relative fluorescence units [RFU]) is monitored over time to produce a real-time fluorescence emergence curve for each reporter dye. The Panther system software compares the fluorescence emergence curves to fixed cut off times to report results (TTime) for HSV-1, HSV-2 and IC.

Kit Components

The Aptima HSV assay is provided as a 100 test kit. The following 3 kits are required to perform the Aptima HSV assay on the Panther system:

- Aptima Herpes Simplex Viruses 1 & 2 Assay Master Kit: Consists of 2 boxes containing eight reagents packaged by storage conditions (1 refrigerated and 1 room temperature box).
- Aptima Herpes Simplex Viruses 1 & 2 Controls Kit: Consists of 1 box containing a Positive and Negative Control.
- Aptima Assay Fluids Kit: This ancillary kit is required to run the assay but is procured separately. The Aptima Assay Fluids Kit consists of 1 box containing 3 components. The ancillary kit previously received market clearance for use in other commercialized Aptima assays (Ref: K003395).

The Aptima HSV assay utilizes two collection and handling kits, which are sold independently of the assay. The collection kits described below have previously received market clearance for use in other commercialized Aptima assays:

- Aptima Multitest Swab Specimen Collection Kit (Ref: K032554)
- Aptima Specimen Transfer Kit (Ref: K063664, K063451, K043224)

Instrumentation

The Aptima HSV assay has been designed for and validated on the Panther system. The Panther system is an integrated hardware and software system that fully automates all steps of nucleic

acid testing necessary for diagnostic assays. The instrument provides automation of all assay steps including sample processing, amplification of nucleic acid, detection and quantitation, amplicon inactivation, and data reduction. The "Panther Instrument" refers to the analyzer and associated hardware combined with the software. The "Panther system" refers to the Panther Instrument operated in conjunction with the Aptima HSV Assay. The assay software in the computer workstation directs the analyzer modules to perform each sequential assay step.

The Panther system was previously FDA cleared for use with other Class II Aptima Assays (Ref: K122062 and K111409).

V. INDICATIONS FOR USE

Intended Use

The Aptima Herpes Simplex Viruses 1 & 2 assay (Aptima HSV 1 & 2 assay) is an *in vitro* diagnostic nucleic acid amplification test (NAAT), using real time transcription-mediated amplification (TMA), for the qualitative detection and differentiation of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) messenger RNA (mRNA) in clinician-collected swab specimens from anogenital skin lesions. The assay is intended for use with swab specimens placed in Aptima specimen transport medium (STM) or in viral transport media (VTM) that is immediately diluted into STM.

The Aptima HSV 1 & 2 assay is intended for use as an aid in the diagnosis of HSV-1 and/or HSV-2 infections in symptomatic male and female patients. The Aptima HSV 1 & 2 assay is indicated for use on the Panther® system.

Warning

The Aptima HSV 1 & 2 assay is not FDA-cleared for use with cerebrospinal fluid (CSF) or for prenatal screening.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

A comparison of the Aptima Herpes HSV assay to the predicate BD ProbeTec Herpes Simplex Viruses (HSV 1 & 2) Q^x Amplified DNA Assays (K103798) is summarized in **Table 1** (similarities) and **Table 2** (differences).

Table 1: Comparison of Similarities Between Aptima HSV Assay and Predicate Device

Item	Aptima HSV Assay (Subject Device) K162673	BD ProbeTec Assay (Predicate Device) K103798
Intended Use	The Aptima Herpes Simplex Viruses 1 & 2 assay (Aptima HSV 1 & 2 assay) is an *in vitro* diagnostic nucleic acid amplification test (NAAT), using real time transcription-mediated amplification (TMA), for the qualitative detection and differentiation of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) messenger RNA (mRNA) in clinician-collected swab specimens from anogenital skin lesions. The assay is intended for use with swab specimens placed in Aptima specimen transport medium (STM) or in viral transport media (VTM) that is immediately diluted into STM. The Aptima HSV 1 & 2 assay is intended for use as an aid in the diagnosis of HSV-1 and/or HSV-2 infections in symptomatic male and female patients. The Aptima HSV 1 & 2 assay is indicated for use on the Panther® system. **Warning** ***The Aptima HSV 1 & 2 assay is not FDA-cleared for use with cerebrospinal fluid (CSF) or for prenatal screening.***	The BD ProbeTec Herpes Simplex Viruses (HSV 1 & 2) QX Amplified DNA Assays, when tested with the BD Viper System in Extracted Mode, use Strand Displacement Amplification technology for the direct, qualitative detection and differentiation of Herpes Simplex virus type 1 (HSV1) and Herpes Simplex virus type 2 (HSV2) DNA in clinician-collected external anogenital lesion specimens. The assays are indicated for use with symptomatic individuals to aid in the diagnosis of anogenital HSV1 and HSV2 infections. ***Warning****:* The BD ProbeTec Herpes Simplex Viruses (HSV 1 & 2) QX Amplified DNA Assays (HSV QX Assays) are not FDA cleared for use with cerebrospinal fluid (CSF). The assays are not intended to be used for prenatal screening or for individuals under the age of 17 years.

Item	Aptima HSV Assay (Subject Device) K162673	BD ProbeTec Assay (Predicate Device) K103798
Qualitative /Quantitative Assay	Qualitative	Qualitative
Controls	Positive and negative controls. Provided as part of the Master Kit.	Per PI, positive and negative controls. Provided as part of a Master Kit.
Patient Population	Symptomatic male and female patients	Symptomatic male and female patients
Specimen Types	Clinician-collected swab specimens from skin lesions in the anogenital region.	Clinician-collected external anogenital lesion specimens.

Table 2: Comparison of Differences Between Aptima HSV Assay and Predicate Device

Item	Aptima HSV Assay (Subject Device)	BD ProbeTec Assay (Predicate Device)
Target	mRNA	DNA
Function	Qualitative detection and differentiation of mRNA from herpes simplex viruses 1 & 2	Qualitative detection and differentiation of DNA from herpes simplex viruses 1 & 2
Technology	The subject assay is comprised of a nucleic acid amplification test (NAAT) for use on the automated Panther system, that utilizes target capture to extract viral mRNA from patient samples, and real-time transcription mediated amplification (TMA) and detection of HSV-1, and HSV-2 mRNAs and an internal control RNA.	The predicate assay is comprised of an amplification test for use on the automated BD Viper System that utilizes non-specific extraction of DNA from clinical specimens, followed by binding of DNA to magnetic particles, washing of the bound nucleic acid and elution in an amplification-compatible buffer. When present, HSV1 and/or 2 DNA is detected by Strand Displacement Amplification (SDA) of type-specific target sequences in the presence of a fluorescently labeled detector probe.

Item	Aptima HSV Assay (Subject Device)	BD ProbeTec Assay (Predicate Device)
Platform	The Panther system is an in vitro diagnostic device intended for use in clinical laboratories by trained laboratory professionals for the automation of NAATs. The Panther system provides automation of all assay steps, including sample processing, amplification of target nucleic acid, amplicon detection, data reduction, and amplicon inactivation.	BD Viper System is an in vitro diagnostic device intended for use in clinical laboratories by trained laboratory professionals for the automation of NAATs. The BD Viper System provides automated non-specific extraction and amplification of DNA from clinical specimens. In Extracted Mode, it uses Strand Displacement Amplification technology. When present, HSV 1 and/or 2 amplicon is detected by Strand Displacement Amplification.
Specimen Collection	• Aptima Multitest Swab Specimen Collection Kit (STM) • Aptima Specimen Transfer Kit (for use with specimens collected in VTM)	• BD ProbeTecQ^{x} Collection Kit for Endocervical or Lesion Specimens • BD Universal Viral Transport Medium (VTM) w/Swab Collection Kit Copan Universal Transport Medium w/Swab Collection Kit (same as BD VTM Kit)

VII. PERFORMANCE DATA

The following performance data were provided in support of the substantial equivalence determination.

Brief Description of Analytical (Non-Clinical) Studies

The following analytical studies (non-clinical) were conducted to support the clearance of the Aptima HSV Assay on the Panther system.

Analytical Sensitivity and Limit of Detection (LoD)

The LoD in clinical specimen matrix was determined by testing dilution panels for each of two strains of HSV-1 virus (Macintyre and HF strains) and two strains of HSV-2 viral (MS and G) in

both Aptima Specimen Transport Media (STM) and Viral Transport Media (VTM) negative clinical pools in the Aptima HSV assay. LoD for the assay was based on results from the reagent lot with the highest concentration for the 95% predicted detection limit. **Table 3** contains the probit-predicted 95% LoD values of the Aptima HSV assay for HSV-1 and HSV-2 in both specimen types.

Table 3: HSV 1 & 2 LoD in VTM and STM

HSV Type/Strain	Specimen Type	LoD TCID50/mL
HSV-1 MacIntyre	STM	60.6
	VTM	186.9
HSV-1 HF	STM	78.9
	VTM	159.3
HSV-2 MS	STM	18.2
	VTM	28.7
HSV-2 G	STM	18.8
	VTM	128.8

Limit of Detection Verification

LoD of the Aptima HSV 1 & 2 assay was verified using two clinical isolates of HSV-1 and two clinical isolates of HSV-2 from HSV-positive clinical specimens that were cultured and quantitated in-house. Each isolate was tested with the Aptima HSV 1 & 2 assay using 60 replicates each at 1X LoD, 3X LoD, and 10X LoD, see Table 4 for LoD values. Testing was completed in both STM and VTM matrix for all four clinical isolates and was conducted using 3 lots of reagents. All replicates for all clinical isolates at all three concentrations tested were detected by the Aptima HSV 1 & 2 assay, demonstrating that the assay can accurately detect a range of both HSV-1 and HSV-2 isolates at the determined LoD.

Table 4: HSV 1 and HSV 2 LoD

HSV Type	Media Type	LoD (TCID50/mL)
HSV-1	STM	78.9
	VTM	186.9
HSV-2	STM	18.8
	VTM	128.8

Limit of Detection in Neat STM.

The LoD in neat STM (Aptima Specimen Transport Media) was determined by testing panels that consisted of dilutions of HSV-1 and HSV-2 in vitro RNA transcripts (IVT) and dilutions of HSV-1 and HSV-2 viral particles in neat STM matrix. Through Probit analysis, results show the Aptima HSV Assay has a predicted 95% LoD of 68.3 copies/mL for HSV-1 IVT and 49.4 copies/mL for HSV-2 IVT. The assay has a predicted 95% LoD of 28.9 $TCID_{50}$/mL for HSV-1 MacIntyre virus and 0.54 $TCID_{50}$/mL for HSV-2 MS virus.

Interfering Substances

Potentially interfering substances listed in Table 4 were tested in the Aptima HSV 1 & 2 assay. Panels were built in STM and evaluated for potential effects on both assay sensitivity and specificity. Sensitivity performance was evaluated separately for both HSV-1 and HSV-2 by spiking viral particles into substance-containing panels at 3X the LoD. HSV-negative panels containing each substance were also evaluated for specificity.

No effect on assay performance was observed in the presence of a representative brand of the following exogenous and endogenous substances tested at the concentrations indicated in Table 5.

Table 5: Potential Interfering Substances

Interfering Substance	Final Concentration
Vaginal lubricant	5% V/V
Spermicide/Contraceptive Jelly	4% W/V
Anti-fungal cream	5% W/V
Douche	5% V/V
Feminine Spray	5% W/V
Body Lotion	5% W/V
Powder	5% W/V
Glacial Acetic Acid Wash Solution	5% V/V
Hemorrhoidal Cream	5% W/V
Urine	5% V/V
Whole Blood	0.5% V/V
Leukocytes	$4x10^5$ Cells/mL
Mucus	0.3% W/V
Seminal Fluid	5% V/V
Feces	0.03% W/V
Protein	4% W/V
Antiviral drug (Acyclovir)	5% W/V

Co-Infection

A co-infection study was evaluated to assess the ability of the Aptima HSV 1 & 2 assay to detect HSV 1 and HSV 2 analytes when both are present in one specimen. Low and high titer concentrations of HSV-1 viral particles in STM were tested in combination with low, moderate, and high concentrations of HSV-2 virus. Panel composition and concentrations are listed in Table 6. All testing resulted in 100% detection for both HSV-1 and HSV-2.

Table 6: Co-infected Panel

Panel Member	HSV-1 Concentration	HSV-2 Concentration
HSV-1 Low; HSV-2 Low	86.7 $TCID_{50}$/mL[1]	1.62 $TCID_{50}$/mL[2]
HSV-1 Low; HSV-2 Moderate	86.7 $TCID_{50}$/mL[1]	540 $TCID_{50}$/mL[3]
HSV-1 High; HSV-2 Low	28,900 $TCID_{50}$/mL[4]	1.62 $TCID_{50}$/mL[2]
HSV-1 High; HSV-2 Moderate	28,900 $TCID_{50}$/mL[4]	540 $TCID_{50}$/mL[3]
HSV-1 Low; HSV-2 High	86.7 $TCID_{50}$/mL[1]	9,000 $TCID_{50}$/mL[5]

[1] 3X LoD HSV-1.
[2] 3X LoD HSV-2.
[3] 1000X LoD HSV-2.
[4] 1000X LoD HSV-1.
[5] 16667X LoD HSV-2.

Cross-Reactivity

A study was performed to evaluate the cross-reactivity and interference with the Aptima HSV 1 & 2 assay in the presence of non-targeted microorganisms that could be present in clinical specimens. A panel consisting of 48 microorganisms (Table 7) was tested in STM at a test concentration of 1 x 10^5 units/mL for viruses and 1 x 10^6 units/mL for all other organisms. Organisms were tested in the absence of HSV or in the presence of either HSV-1 or HSV-2 at 3X LoD. Thirty replicates were tested per panel condition. No evidence of cross-reactivity or microbial interference was observed for any of the 48 test microorganisms tested with the Aptima HSV 1 & 2 assay, at the following concentrations.

Table 7: Cross-Reactivity and Microbial Interference Panel

Microorganism	Concentration
Acinetobacter calcoaceticus	$1x10^6$ CFU/mL[1,2]
Acinetobacter lwoffii	$1x10^6$ CFU/mL[1,2]
Actinomyces israelii	$1x10^6$ RNA copies /mL[2]
Adenovirus type 1	$1x10^5$ TCID50/mL[3]
Alcaligenes faecalis	$1x10^6$ CFU/mL[1]
Atopobium vaginae	$1x10^6$ RNA copies /mL[2]
Bacteroides fragilis	$1x10^6$ CFU/mL[1,2]
Bifidobacterium adolescentis	$1x10^6$ CFU/mL[1,2]
BK virus	$1x10^5$ DNA copies/mL[3]
Bordetella bronchiseptica	$1x10^6$ CFU/mL[1,2]
Bordetella pertussis	$1x10^6$ CFU/mL[1,2]
Campylobacter jejuni	$1x10^6$ CFU/mL[1,2]
Candida glabrata	$1x10^6$ CFU/mL[1,2]
Clostridium difficile	$1x10^6$ CFU/mL[1,2]
Clostridium perfringens	$1x10^6$ CFU/mL[1,2]
Corynebacterium genitalium	$1x10^6$ CFU/mL[1,2]
Cryptococcus neoformans	$1x10^6$ CFU/mL[1,2]
Enterobacter aerogenes	$1x10^6$ CFU/mL[1,2]
Enterobacter cloacae	$1x10^6$ CFU/mL[1,2]
Enterococcus faecium	$1x10^6$ CFU/mL[1,2]
Enterococcus faecalis	$1x10^6$ CFU/mL[1,2]
Epstein-Barr virus	$1x10^5$ DNA copies/mL[3]
Escherichia coli	$1x10^6$ CFU/mL[1,2]
Fusobacterium nucleatum	$1x10^6$ CFU/mL[1,2]
Gardnerella vaginalis	$1x10^6$ CFU/mL[1,2]
Haemophilus ducreyi	$1x10^6$ CFU/mL[1,2]
Hepatitis B virus	$1x10^5$ IU/mL[4,3]
Klebsiella pneumoniae	$1x10^6$ CFU/mL[1,2]
Lactobacillus crispatus	$1x10^6$ CFU/mL[1,2]
Moraxella catarrhalis	$1x10^6$ CFU/mL[1,2]
Mycoplasma hominis	$1x10^6$ RNA copies /mL[2]
Mycoplasma orale	$1x10^6$ RNA copies /mL[2]
Neisseria gonorrhoeae	$1x10^6$ CFU/mL[1,2]
Neisseria meningitidis	$1x10^6$ CFU/mL[1,2]
Parvovirus B19	$1x10^5$ TCID50/mL[3]
Prevotella bivia	$1x10^6$ CFU/mL[1,2]

Table 7: Cross-Reactivity and Microbial Interference Panel

Microorganism	Concentration
Propionibacterium acnes	$1x10^6$ CFU/mL[1,2]
Proteus mirabilis	$1x10^6$ CFU/mL[1,2]
Proteus vulgaris	$1x10^6$ CFU/mL[1,2]
Pseudomonas aeruginosa	$1x10^6$ CFU/mL[1,2]
Staphylococcus aureus	$1x10^6$ CFU/mL[1,2]
Staphylococcus epidermidis	$1x10^6$ CFU/mL[1,2]
Staphylococcus saprophyticus	$1x10^6$ CFU/mL[1,2]
Streptococcus mitis	$1x10^6$ CFU/mL[1,2]
Streptococcus pneumoniae*	$1x10^5$ CFU/mL[1,2]
Streptococcus pyogenes	$1x10^6$ CFU/mL[1,2]
Varicella-zoster virus	$1x10^5$ DNA copies/mL[3]
West Nile virus	$1x10^5$ TCID50/mL[3]

[1]CFU = Colony Forming Units, [2]Procured internally from Hologic, Inc.,[3]Obtained from ZeptoMetrix Corporation (Buffalo NY), [4]IU=International Units
*Cross reactivity was observed in *Streptococcus pneumoniae at* $1x10^6$ CFU/mL

Specimen Inhibition in Clinical Samples

An evaluation was conducted of the whole system failure rate leading to false negative or invalid results by testing low positive specimens. HSV negative VTM and STM samples were spiked with HSV-1 Macintyre or HSV-2 MS strain cultured virus at a test concentration at 3X LoD. Of 201 STM specimens tested, the false negative rate was 0% (0/201; 95% CI: 0.0-1.9) for HSV-1 and 0.5% (1/201; 95% CI: 0.1-2.8) for HSV-2. Of 202 VTM specimens tested, the false negative rate was 0% (0/202; 95% CI: 0.0-1.9) for HSV-1 and 0% (0/202; 95% CI: 0.0-1.9) for HSV-2.

Reproducibility and Within Laboratory Precision

To evaluate the reproducibility and within laboratory precision of the Aptima HSV assay, one HSV negative panel and six HSV positive panels were tested. The six HSV positive panels were spiked with HSV-1 cultured virus strain (MacIntyre) and/or HSV-2 cultured virus strain (MS). All panel members were made using STM. Three replicates of each panel was tested by three operators in two runs each in three reagent lots over 13 days.

Results showed that the Aptima HSV assay has acceptable precision and is reproducible with various instruments, reagent lots, and operators.

Brief Description of Clinical Studies

Clinical testing of the Aptima HSV assay on the Panther system included performance and reproducibility testing. Substantial equivalence is based in part on the performance study.

Reproducibility

Aptima HSV 1 & 2 assay reproducibility was evaluated at three external US sites. Testing was performed using three lots of assay reagents and six operators (two at each site). At each site, testing was performed for at least six days. Panel members were created by spiking HSV-1 and/or HSV-2 viral particles into STM. Final HSV-1 concentrations ranged from 0 $TCID_{50}$/mL to 86.96 $TCID_{50}$/mL and final HSV-2 concentrations ranged from 0 $TCID_{50}$/mL to 1.63 $TCID_{50}$/mL.

HSV-negative panel members and panel members containing low (<1 X LoD and 1-2 X LoD) and moderate levels (2-3 X LoD) of HSV-1 and HSV-2 were tested with the Aptima HSV 1 & 2 assay. Agreement with expected results was 100% for HSV-1 and HSV-2 in the negative and moderate positive panel members and ≤ 100% in panel members with concentrations near or below the 95% LoD of the assay in STM spiked with viral particles (Tables 8). The signal variability of the Aptima HSV 1 & 2 assay in the low and moderate positive panel members and in the positive control is summarized in Tables 9 and 10.

Table 8. Reproducibility - Agreement of Aptima HSV 1 & 2 Assay with Expected Results

Conc		Target Conc ($TCID_{50}$/mL)		Expected Result			Agreed (n)		Agreement (%) (95% CI)	
HSV-1	**HSV-2**	**HSV-1**	**HSV-2**	**HSV-1**	**HSV-2**	**N**	**HSV-1**	**HSV-2**	**HSV-1**	**HSV-2**
Neg	Neg	0	0	Neg	Neg	108	108	108	100 (96.6-100)	100 (96.6-100)
LPos	Neg	28.90	0	Pos	Neg	108	103	108	95.4 (89.6-98.0)	100 (96.6-100)
Neg	LPos	0	0.54	Neg	Pos	108	108	105	100 (96.6-100)	97.2 (92.1-99.1)
LPos	MPos	28.90	1.63	Pos	Pos	108	97	108	89.8 (82.7-94.2)	100 (96.6-100)
MPos	LPos	86.96	0.54	Pos	Pos	108	108	108	100 (96.6-100)	100 (96.6-100)
HNeg	Neg	3.00	0	Pos	Neg	108	50	108	46.3 (37.2-55.7)	100 (96.6-100)
Neg	HNeg	0	0.20	Neg	Pos	108	108	86	100 (96.6-100)	79.6 (71.1-86.1)

CI = confidence interval, Conc = concentration, HNeg = high negative (<1 X LoD), LPos = low positive (1-2 X LoD), MPos = moderate positive (2-3 X LoD), Neg = negative, Pos = positive

Table 9. Reproducibility - Signal Variability of the Aptima HSV 1 & 2 Assay

Virus Conc	N	Mean TTime	Between Sites SD (%CV)	Between Lots SD (%CV)	Between Operators/ Days[1] SD (%CV)	Between Runs SD (%CV)	Within Runs SD (%CV)	Total SD (%CV)
HSV-1								
LPos	103	24.68	0 (0)	1.63 (6.62)	0.75 (3.04)	0.54 (2.18)	0.88 (3.55)	2.07 (8.40)
LPos	97	23.91	0 (0)	2.18 (9.11)	0.86 (3.58)	0 (0)	1.60 (6.71)	2.84 (11.87)
MPos	108	22.96	0 (0)	1.54 (6.69)	0.38 (1.65)	0.68 (2.96)	0.94 (4.11)	1.96 (8.55)
HSV-2								
LPos	105	25.49	0 (0)	0.84 (3.30)	0.70 (2.74)	0 (0)	2.52 (9.87)	2.74 (10.76)
LPos	108	25.34	0 (0)	1.54 (6.08)	0.86 (3.41)	0.59 (2.34)	2.67 (10.53)	3.26 (12.85)
MPos	108	22.91	0 (0)	1.09 (4.76)	0.35 (1.53)	0.42 (1.83)	1.06 (4.64)	1.62 (7.07)

Conc = concentration, CV = coefficient of variation, LPos = low positive (1-2 X LoD), MPos = moderate positive (2-3 X LoD), SD = standard deviation

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, SD and CV are shown as 0.

[1]Between Operators may be confounded with Between Days; therefore, Between Operators and Between Days estimates are combined in Between Operators/Days.

Table 10. Reproducibility - Signal Variability of the Aptima HSV 1 & 2 Assay in the Positive Control

Virus	N	Mean TTime	Between Sites SD (%CV)	Between Lots SD (%CV)	Between Operators/Days[1] SD (%CV)	Within Days SD (%CV)	Total SD (%CV)
HSV-1	36	20.11	0 (0)	1.30 (6.48)	0.40 (1.99)	1.09 (5.42)	1.75 (8.68)
HSV-2	36	22.16	0 (0)	1.61 (7.27)	0.71 (3.21)	1.38 (6.22)	2.24 (10.09)

CV = coefficient of variation, SD = standard deviation

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, SD and CV are shown as 0.

[1]Between Operators may be confounded with Between Days; therefore, Between Operators and Between Days estimates are combined in Between Operators/Days.

Clinical Performance Study

A prospective, multicenter clinical study was conducted to establish the performance characteristics of the Aptima HSV 1 & 2 assay. A total of 544 evaluable subjects (195 males and 349 females) with active anogenital lesions were enrolled from 17 US clinical sites, including family planning, dermatology, pediatrics/adolescent, sexually transmitted infection, private practice, public health clinics, hospitals, universities, and clinical research sites.

Two (2) swab specimens were collected from a single lesion from each subject: one was collected with a swab from a commercially available VTM collection kit and one was collected with a swab from the Aptima Multitest Swab Specimen Collection Kit (STM). Specimens were processed in accordance with the appropriate package insert instructions. Aptima HSV 1 & 2 assay testing was performed at 3 external sites. The performance of the Aptima HSV-1 & 2 assay was evaluated relative to a composite reference. The composite reference method used the results from ELVIS HSV ID and D^3 Typing Test system viral culture and a validated bidirectional PCR/sequencing procedure. A third FDA-cleared assay for HSV-1 and HSV-2, was used to determine the final composite reference interpretation when the ELVIS D^3 culture and PCR/sequencing results did not agree on the type of HSV detected or when PCR/sequencing detected both HSV-1 and HSV-2. The performance of the Aptima HSV 1 & 2 assay was estimated for detection of HSV-1 and HSV-2 separately using each specimen type.

For HSV-1, of the 544 evaluable subjects, 528 VTM and 531 STM specimen types resulted in evaluable results for HSV-1 analysis (16 VTM and 13 STM were reported as unknown composite reference interpretation or withdrawn).

For HSV-2, of the 544 evaluable subjects, 533 VTM and 535 STM specimen types produced evaluable results for HSV-2 analysis (11 VTM and 9 STM were reported as unknown composite reference interpretation or withdrawn).

The performance of Aptima HSV 1 & 2 assay compared to the composite reference method is summarized for anogenital skin lesions in STM and VTM in Tables 11 and 12. Overall, for HSV-1, sensitivity was 93.4% for VTM and 94.7% for STM specimen types and specificity was 99.8% for VTM and 99.6% for STM specimen types (Table 11). For HSV-2, sensitivity was

96.9% for VTM and 98.4% for STM specimen types and specificity was 97.5% for VTM and 92.8% for STM specimen types (Table 12).

Table 11. Summary of HSV-1 Results by VTM and STM Specimen Type

Specimen Type	Gender	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	Combined	528	71	1[3]	451	5[4]	14.4	93.4 (85.5-97.2)	99.8 (98.8->99.9)	98.6 (93.0-100)	98.9 (97.6-99.6)
	Male	192	19	1	170	2	10.9	90.5 (71.1-97.3)	99.4 (96.8-99.9)	95.0 (78.6-99.8)	98.8 (96.4-99.9)
	Female	336	52	0	281	3	16.4	94.5 (85.1-98.1)	100 (98.7-100)	100 (93.7-100)	98.9 (97.1-99.8)
STM	Combined	531	71	2[5]	454	4[6]	14.1	94.7 (87.1-97.9)	99.6 (98.4-99.9)	97.3 (91.1-99.6)	99.1 (97.9-99.8)
	Male	192	20	2	169	1	10.9	95.2 (77.3-99.2)	98.8 (95.8-99.7)	90.9 (74.5-98.7)	99.4 (97.2-100)
	Female	339	51	0	285	3	15.9	94.4 (84.9-98.1)	100 (98.7-100)	100 (93.6-100)	99.0 (97.2-99.8)

STM = Aptima Multitest Swab specimen, Prev = prevalence, VTM = VTM sample

[1]Score CI

[2]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

[3]The sample had a negative culture result.

[4]Two samples had negative culture results, one had a non-typable HSV positive culture result, and two were HSV-1 positive by culture.

[5]Both specimens had negative culture results.

[6]One specimen had a negative culture result, one had a non-typable HSV positive culture result, and two were HSV-1 positive by culture.

Table 12. Summary of HSV-2 Results by VTM and STM Specimen Type

Specimen Type	Gender	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	Combined	533	248	7[3]	270	8[4]	48.0	96.9 (94.0-98.4)	97.5 (94.9-98.8)	97.3 (94.7-98.8)	97.1 (94.6-98.7)
	Male	194	79	2	110	3	42.3	96.3 (89.8-98.7)	98.2 (93.7-99.5)	97.5 (92.0-99.7)	97.3 (93.0-99.4)
	Female	339	169	5	160	5	51.3	97.1 (93.5-98.8)	97.0 (93.1-98.7)	97.1 (93.8-99.0)	97.0 (93.4-99.0)
STM	Combined	535	253	20[5]	258	4[6]	48.0	98.4 (96.1-99.4)	92.8 (89.1-95.3)	92.7 (89.4-95.3)	98.5 (96.3-99.6)
	Male	194	79	6	106	3	42.3	96.3 (89.8-98.7)	94.6 (88.8-97.5)	92.9 (86.5-97.1)	97.2 (92.8-99.4)
	Female	341	174	14	152	1	51.3	99.4 (96.8-99.9)	91.6 (86.3-94.9)	92.6 (88.5-95.7)	99.3 (96.6-100)

STM = Aptima Multitest swab specimen, Prev = prevalence, VTM = VTM sample

[1]Score CI

[2]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

[3]Six samples had negative culture results and one was HSV-1 positive by culture.

[4]All eight samples had negative culture results.

[5]Eighteen specimens had negative culture results and two were HSV-1 positive by culture.

[6]All four specimens had negative culture results.

Expected Values

Prevalence

The prevalence of HSV-1 and HSV-2 observed during a multi-center clinical study was calculated for the Aptima HSV 1 & 2 assay. The prevalence of HSV-1 and HSV-2 with the Aptima HSV 1 & 2 assay is summarized by age group, gender group, and specimen type in Table 13.

Table 13. Gender and Age Distribution by VTM and STM Specimen Type[1]

Gender / Age Group	%Prevalence (# positive results/# tested)			
	VTM		STM	
	HSV-1	HSV-2	HSV-1	HSV-2
Male				
All ages	10.4 (20/192)	41.8 (81/194)	11.5 (22/192)	43.8 (85/194)
<2 years	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)
2 to 11 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
12 to 21 years	11.1 (2/18)	38.9 (7/18)	11.1 (2/18)	38.9 (7/18)
22 to 30 years	14.0 (13/93)	34.0 (32/94)	14.9 (14/94)	35.8 (34/95)
31 to 40 years	12.5 (5/40)	50.0 (20/40)	12.8 (5/39)	53.8 (21/39)
41 to 50 years	0.0 (0/20)	52.4 (11/21)	0.0 (0/20)	52.4 (11/21)
51 to 60 years	0.0 (0/14)	57.1 (8/14)	7.1 (1/14)	57.1 (8/14)
> 60 years	0.0 (0/6)	50.0 (3/6)	0.0 (0/6)	66.7 (4/6)
Female				
All ages	15.5 (52/336)	51.3 (174/339)	15.0 (51/339)	55.1 (188/341)
<2 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
2 to 11 years	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)
12 to 21 years	23.8 (15/63)	56.3 (36/64)	23.1 (15/65)	59.1 (39/66)
22 to 30 years	14.6 (25/171)	52.6 (90/171)	14.5 (25/172)	56.4 (97/172)
31 to 40 years	11.1 (7/63)	46.0 (29/63)	12.5 (8/64)	47.6 (30/63)
41 to 50 years	18.2 (4/22)	39.1 (9/23)	10.0 (2/20)	47.6 (10/21)
51 to 60 years	7.7 (1/13)	46.2 (6/13)	7.1 (1/14)	57.1 (8/14)
> 60 years	0.0 (0/3)	100 (4/4)	0.0 (0/3)	100 (4/4)
Combined				
All ages	13.6 (72/528)	47.8 (255/533)	13.7 (73/531)	51.0 (273/535)
<2 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
2 to 11 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
12 to 21 years	21.0 (17/81)	52.4 (43/82)	20.5 (17/83)	54.8 (46/84)
22 to 30 years	14.4 (38/264)	46.0 (122/265)	14.7 (39/266)	49.1 (131/267)
31 to 40 years	11.7 (12/103)	47.6 (49/103)	12.6 (13/103)	50.0 (51/102)
41 to 50 years	9.5 (4/42)	45.5 (20/44)	5.0 (2/40)	50.0 (21/42)
51 to 60 years	3.7 (1/27)	51.9 (14/27)	7.1 (2/28)	57.1 (16/28)
> 60 years	0.0 (0/9)	70.0 (7/10)	0.0 (0/9)	80.0 (8/10)

[1]No subjects had positive Aptima HSV 1 & 2 assay results for both HSV-1 and HSV-2.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive and negative predictive values (PPV and NPV) of the Aptima HSV 1 & 2 assay for detection of HSV-1 and HSV-2 across different hypothetical prevalence rates are shown for each specimen type in Table 14. These calculations are based on the overall estimated sensitivity and specificity for each specimen type as determined in the clinical performance study.

Table 14. Prevalence vs Hypothetical PPV[1] and NPV[2] for Detection of HSV-1 and HSV-2 by Specimen Type

		HSV-1		HSV-2	
Specimen Type	**Prevalence (%)**	**PPV (%)**	**NPV (%)**	**PPV (%)**	**NPV (%)**
VTM	1	81.0	99.9	27.9	100
	2	89.6	99.9	43.9	99.9
	5	95.7	99.7	66.9	99.8
	10	97.9	99.3	81.0	99.6
	20	99.1	98.4	90.6	99.2
	30	99.5	97.3	94.3	98.6
	40	99.6	95.8	96.2	97.9
	50	99.8	93.8	97.5	96.9
STM	1	68.6	99.9	12.1	100
	2	81.5	99.9	21.8	100
	5	91.9	99.7	41.9	99.9
	10	96.0	99.4	60.3	99.8
	20	98.2	98.7	77.4	99.6
	30	98.9	97.8	85.4	99.3
	40	99.3	96.6	90.1	98.9
	50	99.5	94.9	93.2	98.4

STM = Aptima Multitest swab specimen, VTM = VTM sample

[1]PPV was calculated using:
(Sensitivity x Prevalence) / (Sensitivity x Prevalence + [1 - Specificity] x [1 - Prevalence]).

[2]NPV was calculated using:
(Specificity x [1 - Prevalence]) / ([1 - Sensitivity] x Prevalence + Specificity x [1 - Prevalence]).

TTime Distribution for Aptima HSV 1 & 2 Assay Positive Controls

The distribution of the TTime values for the Aptima HSV 1 & 2 assay positive control from all valid Aptima HSV 1 & 2 assay runs performed during the clinical performance study is presented in Table 15.

Table 15. Distribution of TTimes for Aptima HSV 1 & 2 Assay Positive Controls

	TTime	
Statistics	**HSV-1**	**HSV-2**
N	107	107
Mean	20.03	22.01
Median	19.8	21.7
SD	1.198	1.612
CV (%)	6.0	7.3
Minimum	18.1	19.5
Maximum	22.9	26.2

CV = coefficient of variation, SD = standard deviation

VIII. CONCLUSIONS

The analytical and clinical study results demonstrate that the Aptima HSV assay on the Panther system is substantially equivalent to the predicate device that is currently marketed for the same intended use. Hardware and software verification and validation demonstrate that the Aptima HSV assay on the Panther system will perform as intended.