May 23, 2019

Hologic, Inc.
Jeffrey Hergesheimer
Regulatory Affairs Manager
10210 Genetic Center Drive
San Diego, California 92121

Re: K190452
Trade/Device Name: Aptima BV Assay, Aptima BV Controls Kit, Aptima BV Calibrator Kit
Regulation Number: 21 CFR 866.3975
Regulation Name: Device that detects nucleic acid sequences from microorganisms associated with vaginitis and bacterial vaginosis.
Regulatory Class: Class II
Product Code: PQA, NSU, PMN
Dated: February 22, 2019
Received: February 25, 2019

Dear Jeffrey Hergesheimer:

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. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. 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) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/CombinationProducts/GuidanceRegulatoryInformation/ucm597488.htm); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

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 comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

for

Uwe Scherf, Ph.D.
Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

510(k) SUMMARY
Aptima BV Assay

I. SUBMITTER

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

Contact Person: Jeffrey Hergesheimer, MS, RAC
Regulatory Affairs Manager
Jeffrey.Hergesheimer@Hologic.com
Phone: (858) 410-8536
Fax: (858) 410-5557

Date Prepared: February 19, 2019

II. DEVICE

Proprietary Name of Device:	Aptima BV Assay
Classification Name:	Device that detects nucleic acid sequences from microorganisms associated with vaginitis and bacterial vaginosis
Regulation Number:	21 CFR 866.3975
Regulatory Class:	Class II
Product Code:	PQA, NSU, PMN

III. PREDICATE DEVICE

The predicate device is the BD MAX Vaginal Panel (DEN160001; approved 10/28/2016, BD Diagnostics).

IV. DEVICE DESCRIPTION

The Aptima BV assay is an in vitro nucleic acid amplification test for the detection and quantitation of rRNA from bacteria associated with bacterial vaginosis in women with a clinical presentation consistent with vaginitis/vaginosis. The Aptima BV assay utilizes the automated Panther system to provide qualitative results to aid in the diagnosis of bacterial vaginosis.

Principles of the Procedure

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

Specimens are collected in a tube containing specimen transport media (STM) that lyses the cells, releases the RNA, and protects it from degradation during storage. When the Aptima BV assay is performed, capture oligonucleotides hybridize to highly conserved regions of the target RNA, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

Target amplification occurs via TMA, a transcription-based nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target RNA sequence, adding a promoter sequence for T7 RNA polymerase. 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 when the torch is not hybridized to the amplicon. When the torch binds to the amplicon, the fluorophore is separated from the quencher and emits a signal at a specific wavelength when excited by a light source. The Panther system detects and discriminates between four fluorescent signals corresponding to *Lactobacillus* group, *Atopobium vaginae*, *Gardnerella vaginalis* and IC amplification products. The Panther system software compares signal emergence times for each target organism to calibration information in order to determine the BV Positive or Negative status of each sample.

Assay Components

The Aptima BV assay is provided as a 100-test kit. The Aptima BV assay master kit contains 8 reagents, 1 calibrator, and 2 controls required for sample processing. There are 4 boxes that make up the assay master kit. Boxes 1 and 2 contain the Aptima BV assay reagents packaged according to storage conditions. Box 3 contains the calibrator, and Box 4 contains the controls when provided as part of the master kit. The Aptima BV Calibrator and Controls kit may also be procured separately if customers need additional calibrators or controls. A listing of the components that are required to perform the Aptima BV assay are detailed in **Table 1.** In addition, there is one ancillary kit required to run the assay, and one collection kit utilized for collection of specimens (**Table 2**).

Table 1: Reagents Required to Perform the Aptima BV Assay

Box	Components Description
1	Amplification Reagent
	Enzyme Reagent
	Promoter Reagent
	Internal Control
2	Amplification Reconstitution Solution
	Enzyme Reconstitution Solution
	Promoter Reconstitution Solution
	Target Capture Reagent
3	Positive Calibrator
4	Negative Control
	Positive Control

Table 2: Ancillary and Collection Kits Required to Perform the Aptima BV Assay

Aptima Assay Fluids Kit
Aptima Multitest Swab Specimen Collection Kit

Instrumentation

The Aptima BV assay has been designed for and validated on the Panther system. The Panther system is an integrated hardware and software system that together with the Aptima BV assay fully automates all the steps necessary to perform the assay from sample preparation through amplification of nucleic acid, detection, data reduction and amplicon inactivation.

V. INDICATIONS FOR USE

The Aptima BV assay is an *in vitro* nucleic acid amplification test that utilizes real time transcription-mediated amplification (TMA) for detection and quantitation of ribosomal RNA from bacteria associated with bacterial vaginosis (BV), including *Lactobacillus* (*L. gasseri, L. crispatus,* and *L. jensenii*), *Gardnerella vaginalis,* and *Atopobium vaginae*. The assay reports a qualitative result for BV and does not report results for individual organisms. The assay is intended to aid in the diagnosis of BV on the automated Panther system using clinician-collected and patient-collected vaginal swab specimens from females with a clinical presentation consistent with vaginitis and/or vaginosis.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

A comparison of the Aptima BV assay to the predicate device, BD MAX Vaginal Panel, is summarized in **Table 4** (similarities) and **Table 5** (differences).

Table 4: Similarities Between Predicate Device and Subject Device

Item	BD MAX Vaginal Panel (DEN160001)	Aptima BV Assay (Subject Device)
Patient Population	Women with a clinical presentation of vaginitis/vaginosis	Same
Specimen Types	Vaginal swabs in patients who are symptomatic for vaginitis/vaginosis	Same
Assay Controls	Incorporates an Internal Control in every test. Uses external positive and negative controls.	Same

Table 5: Differences Between Predicate Device and Subject Device

Item	BD MAX Vaginal Panel (DEN160001)	Aptima BV Assay (Subject Device)
Organisms Detected	*Lactobacillus* (*L. crispatus,* and *L. jensenii*), *Lactobacillus* (*L. gasseri, L. crispatus,* and *L. jensenii*), *Atopobium vaginae,* Bacterial Vaginosis Associated Bacteria-2 (BVAB-2), *Megasphaera-1, Candida (C. albicans, C. tropicalis, C. parapsilosis, C.*	*Lactobacillus* (*L. gasseri, L. crispatus,* and *L. jensenii*), *Gardnerella vaginalis,* and *Atopobium vaginae*

Item	BD MAX Vaginal Panel (DEN160001)	Aptima BV Assay (Subject Device)
	dubliniensis), Candida glabrata, Candida krusei, Trichomonas vaginalis	
Platform/Technology Principle of Operation	BD MAX System/ Real-time polymerase chain reaction (PCR)	Panther System/ Real-time Transcription Mediated Amplification (TMA)
Analyte	DNA	ribosomal RNA
Assay Calibrators	None	Positive Calibrator
Intended Use	The BD MAX Vaginal Panel performed on the BD MAX System is an automated qualitative *in vitro* diagnostic test for the direct detection of DNA targets from bacteria associated with bacterial vaginosis (qualitative results reported based on detection and quantitation of targeted organism markers), *Candida* species associated with vulvovaginal candidiasis, and *Trichomonas vaginalis* from vaginal swabs in patients who are symptomatic for vaginitis/vaginosis. The test utilizes real-time polymerase chain reaction (PCR) for the amplification of specific DNA targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA from: • Bacterial vaginosis markers (Individual markers not reported) *Lactobacillus* spp. *(L. crispatus* and *L. jensenii) Gardnerella vaginalis* *Atopobium vaginae* Bacterial Vaginosis Associated Bacteria-2 (BVAB-2) *Megasphaera-1* • *Candida* spp. *(C. albicans, C.*	The Aptima BV assay is an *in vitro* nucleic acid amplification test that utilizes real time transcription-mediated amplification (TMA) for detection and quantitation of ribosomal RNA from bacteria associated with bacterial vaginosis (BV), including *Lactobacillus* (*L. gasseri*, *L. crispatus,* and *L. jensenii*), *Gardnerella vaginalis*, and *Atopobium vaginae*. The assay reports a qualitative result for BV and does not report results for individual organisms. The assay is intended to aid in the diagnosis of BV on the automated Panther system using clinician-collected and patient-collected vaginal swab specimens from females with a clinical presentation consistent with vaginitis and/or vaginosis.

Item	BD MAX Vaginal Panel (DEN160001)	Aptima BV Assay (Subject Device)
	tropicalis, C. parapsilosis, C. dubliniensis) • *Candida glabrata* • *Candida krusei* • *Trichomonas vaginalis* The BD MAX Vaginal Panel is intended to aid in the diagnosis of vaginal infections in women with a clinical presentation consistent with bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis.	

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 (non-clinical) studies were conducted to support the clearance of the Aptima BV assay on the Panther system.

Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LoD) and BV positivity limits of the Aptima BV assay were determined by testing a series of panels consisting of *L. crispatus, L. gasseri, L. jensenii, G. vaginalis,* or *A. vaginae* cell lysates diluted into simulated vaginal swab matrix (SVSM). A minimum of 20 replicates of each panel member were tested with each of two reagent lots for a minimum of 40 replicates per panel member. The predicted detection limits for each organism calculated using Probit analysis are shown in **Table 6**.

Table 6: Limit of Detection of the Aptima BV Assay

Organism	Predicted Detection Limit	CFU/mL
A. vaginae	95%	290[1]
G. vaginalis	95%	55[1]
L. crispatus	95%	143
L. gasseri	95%	2,207
L. jensenii	95%	10

[1] Predicted BV Positivity Limits (C_{95}) for *A. vaginae* and *G. vaginalis* in the Aptima BV assay are approximately 5.10 log CFU/mL and 4.86 log CFU/mL, respectively.

Analytical Inclusivity

Five strains of each target organism were tested using lysate targeting 3X C_{95} for *G. vaginalis* and *A. vaginae*, and 3X LoD for Lactobacillus species (*L. crispatus*, *L. gasseri*, and *L. jensenii*) in SVSM. The Aptima BV Assay was BV positive for all five strains of *G. vaginalis* and *A. vaginae* at 3X C_{95}. All five strains of *L. crispatus* and *L. gasseri* were detected at 3X LoD. Three of the five strains of *L. jensenii* were detected at 3X LoD, and the remaining two strains at 10X LoD.

Cross-Reactivity and Microbial Interference

Cross-reactivity and microbial interference with the Aptima BV assay was evaluated in the presence of non-targeted organisms. A panel consisting of 62 organisms (**Table 7**) was tested in SVSM in the absence or in the presence of *L. crispatus* at 3X LoD, *G. vaginalis* at 3X C_{95}, or *A. vaginae* at 3X C_{95}. No cross-reactivity or microbial interference was observed for any of the 62 organisms tested in the Aptima BV assay at the following concentrations.

Table 7: Cross-Reactivity and Microbial Interference Panel

Microorganism	Concentration	Microorganism	Concentration
Acinetobacter lwoffii	$1x10^6$ CFU/mL	Herpes simplex virus I	$1x10^4$ TCID50/mL
Actinomyces israelii	$1x10^6$ CFU/mL	Herpes simplex virus II	$1x10^4$ TCID50/mL
Alcaligenes faecalis	$1x10^6$ CFU/mL	HIV	$1x10^5$ copies/mL
Atopobium minutum	$1x10^6$ CFU/mL	*Klebsiella pneumoniae*	$1x10^6$ CFU/mL
Atopobium parvulum	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^3$ CFU/mL[2]
Atopobium rimae	$1x10^6$ CFU/mL	*Lactobacillus iners*	$1x10^6$ CFU/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Lactobacillus mucosae*	$1x10^6$ CFU/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Leptotrichia buccalis*	$1x10^6$ CFU/mL
Bifidobacterium breve	$1x10^6$ CFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
BVAB-1[1]	$1x10^6$ copies/mL	*Megasphaera Type 1*[1]	$1x10^6$ copies/mL
BVAB-2[1]	$1x10^6$ copies/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Campylobacter jejuni	$1x10^6$ CFU/mL	*Mycoplasma genitalium*	$1x10^6$ CFU/mL
Candida albicans	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^6$ CFU/mL
Candida dubliniensis	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Candida glabrata	$1x10^6$ CFU/mL	*Pentatrichomonas hominis*	$1x10^5$ cells/mL
Candida krusei	$1x10^6$ CFU/mL	*Peptostreptococcus magnus*	$1x10^6$ CFU/mL
Candida lusitaniae	$1x10^6$ CFU/mL	*Pichia fermentans*	$1x10^6$ CFU/mL
Candida orthopsilosis	$1x10^6$ CFU/mL	*Prevotella bivia*	$1x10^6$ CFU/mL
Candida parapsilosis	$1x10^6$ CFU/mL	*Propionibacterium acnes*	$1x10^6$ CFU/mL
Candida tropicalis	$1x10^6$ CFU/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Chlamydia trachomatis	$1x10^6$ IFU/mL	SiHa cells	$1x10^4$ cells/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Sneathia amnii*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Eggerthella lenta	$1x10^6$ CFU/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Treponema pallidum*[1]	$1x10^6$ copies/mL
Escherichia coli	$1x10^6$ CFU/mL	*Trichomonas tenax*	$1x10^5$ cells/mL
Fusobacterium nucleatum	$1x10^6$ CFU/mL	*Trichomonas vaginalis*	$1x10^5$ cells/mL
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Ureaplasma parvum*	$1x10^6$ CFU/mL
HeLa cells	$1x10^4$ cells/mL	*Ureaplasma urealyticum*	$1x10^6$ CFU/mL

CFU = Colony Forming Units; IFU = Inclusion Forming Units; TCID50 = Median Tissue Culture Infectious Dose

[1] In Vitro Transcript tested.

[2] *Lactobacillus acidophilus* affects BV positivity at $1x10^4$ CFU/mL or higher.

Interference

Potentially interfering substances were tested in the Aptima BV assay. Panels were built in SVSM and evaluated for potential effects on assay sensitivity and specificity. Sensitivity performance was evaluated separately for *L. crispatus* by spiking lysate at 3X LoD, and for *G. vaginalis* and *A. vaginae* by spiking lysate at 3X C95. Negative panels containing each substance were also evaluated for specificity. No interference was observed in the presence of the following exogenous and endogenous substances tested at the concentrations listed in **Table 8.**

Table 8: Interfering Substances Panel

Substance	Final Concentration[1]
Whole Blood	5% V/V
Leukocytes	$1x10^6$ cells/mL
Mucus[2]	1.5% V/V
Seminal Fluid	5% V/V
Contraceptive Foam	5% W/V
Contraceptive Film	5% W/V
Tioconazole[3]	1% W/V
Douche	5% W/V
Progesterone	5% W/V
Estradiol	5% W/V
Acyclovir	5% W/V
Metronidazole	5% W/V
Hemorrhoidal Cream	5% W/V
Vaginal Moisturizing Gel[4]	0.4% W/V
Lubricant	5% V/V
Spermicide	5% W/V
Anti-fungal	5% W/V
Deodorant/Spray	5% W/V
Glacial Acetic Acid	5% V/V
Vagisil Cream	5% W/V

W/V = weight by volume; V/V = volume by volume

[1] Final Concentration represents final concentration in the sample when tested on the Panther instrument.

[2] Interference was observed with Mucus at ≥2% V/V and not observed at 1.5% V/V.

[3] Interference was observed with Tioconazole 6.5% Ointment at 5% W/V and not observed at 1% W/V.

[4] Interference was observed with Vaginal Moisturizing Gel at ≥0.5% W/V and not observed at 0.4% W/V.

Within Laboratory Precision

Within Lab Precision was evaluated on three Panther systems at one site. Three operators performed testing across 21 days and three reagent lots. Each operator performed two runs

per day using an 11 member panel. Each run consisted of three replicates of each panel member. The panel members were made using SVSM negative for *Lactobacillus* species, *G. vaginalis*, and *A. vaginae*. Ten panel members contained cell lysates of at least 1 of the following organisms: *L. crispatus*, *L. jensenii*, *G. vaginalis*, or *A. vaginae*; different bacterial combinations were prepared to represent the variety of targeted BV organism combinations present in vaginal specimens. Ten panel members targeted BV Negative (<5% BV Positive), BV High Negative (20-80% BV positive), BV Low Positive (□95% BV positive) and BV Moderate Positive (100% BV positive) results. One negative panel member contained matrix with no added target analytes.

BV percent positive results for each panel are presented in **Table 9**. Signal variability (TTime) of the Aptima BV assay was calculated for each target in analyte positive panel members. Variability calculated between operators, between instruments, between days, between lots, between runs, within run, and overall, is shown in **Tables 10** through **12**.

Table 9: BV Positivity of Precision Panels

Panel Description	BV Positive/ Total n	Expected BV Positivity	BV Positivity (95% CI)
SVSM	0/168	0%	0 (0.0-1.6)
L. crispatus, A. vaginae BV Negative	0 /168	<5%	0 (0.0-1.6)
L. crispatus, G. vaginalis BV High Negative	76 /168	20-80%	45.2 (37.9-52.8)
L. crispatus, G. vaginalis, A. vaginae BV High Negative	131/165[1]	20-80%	79.4 (72.6-84.9)
G. vaginalis BV Low Positive	168/168	≥95%	100 (98.4-100.0)
A. vaginae BV Low Positive	168/168	≥95%	100 (98.4-100.0)
L. jensenii, A. vaginae BV Low Positive	168/168	≥95%	100 (98.4-100.0)
G. vaginalis, A. vaginae BV Low Positive	168/168	≥95%	100 (98.4-100.0)
L. crispatus, G. vaginalis, A. vaginae BV Low Positive	168/168	≥95%	100 (98.4-100.0)
G. vaginalis BV Mod Positive	168/168	100%	100 (98.4-100.0)
A. vaginae BV Mod Positive	168/168	100%	100 (98.4-100.0)

[1] Three invalid results were excluded from the analysis.

Table 10: Signal Variability of Lactobacillus Panel Members

			Between Operators		Between Instruments		Between Days		Between Lots		Between Runs		Within Run		Total	
Panel Description	**N**	**Mean TTime[1]**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
L. crispatus BV Negative[2]	168	19.87	0.10	0.49	0.16	0.80	0.14	0.71	1.03	5.18	0.17	0.09	0.18	0.93	1.08	5.46
L. crispatus BV High Negative[2]	168	23.95	0.11	0.47	0.12	0.52	0.19	0.79	1.22	5.11	0.18	0.77	0.28	1.15	1.29	5.40
L. crispatus BV High Negative[3]	165[4]	22.40	0.09	0.40	0.17	0.74	0.20	0.87	1.22	5.47	0.09	0.39	0.27	1.21	1.29	5.74
L. jensenii BV Low Positive[2]	168	24.80	0.10	0.38	0.14	0.57	0.14	0.57	1.33	5.35	0.17	0.69	0.25	1.01	1.38	5.56
L. crispatus BV Low Positive[3]	168	23.51	0.15	0.63	0.09	0.40	0.17	0.73	1.36	5.77	0.10	0.44	0.31	1.31	1.42	6.02

CV = Coefficient of variation

[1] TTime is shown for *Lactobacillus only*.

[2] Panel member contains 2 different organisms: results are shown for only the *Lactobacillus* component.

[3] Panel member contains 3 different organisms: results are shown for only the *Lactobacillus* component.

[4] Three invalid results were excluded from the analysis.

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 0.00.

Table 11: Signal Variability of G. vaginalis Panel Members

			Between Operators		Between Instruments		Between Days		Between Lots		Between Runs		Within Run		Total	
Panel Description	**N**	**Mean TTime[1]**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
G. vaginalis BV High Negative[2]	168	17.11	0.00	0.00	0.18	1.08	0.17	0.99	0.47	2.75	0.17	0.96	0.16	0.94	0.58	3.39
G. vaginalis BV High Negative[3]	165[4]	15.71	0.00	0.00	0.19	1.19	0.18	1.12	0.48	3.05	0.11	0.72	0.12	0.79	0.57	3.62
G. vaginalis BV Low Positive	168	15.80	0.00	0.00	0.16	1.00	0.14	0.89	0.43	2.70	0.15	0.97	0.15	0.92	0.52	3.30
G. vaginalis BV Mod Positive	168	14.46	0.00	0.00	0.17	1.18	0.05	0.35	0.38	2.63	0.16	1.09	0.18	1.25	0.48	3.35
G. vaginalis BV Low Positive[2]	168	15.01	0.00	0.00	0.14	0.93	0.14	0.91	0.40	2.67	0.16	1.08	0.13	0.86	0.49	3.28
G. vaginalis BV Low Positive[3]	168	14.06	0.00	0.00	0.16	1.11	0.15	1.09	0.39	2.75	0.14	0.99	0.16	1.16	0.49	3.51

CV = Coefficient of variation, Mod = moderate

[1] TTime is shown for *G. vaginalis* only.

[2] Panel member contains 2 different organisms: results are shown for only the *G. vaginalis* component.

[3] Panel member contains 3 different organisms: results are shown for only the *G. vaginalis* component.

[4] Three invalid results were excluded from the analysis.

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 0.00.

Table 12: Signal Variability of A. vaginae Panel Members

			Between Operators		Between Instruments		Between Days		Between Lots		Between Runs		Within Run		Total	
Panel Description	**N**	**Mean TTime[1]**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
A. vaginae BV Negative[2]	168	18.20	0.02	0.11	0.25	1.36	0.15	0.84	0.58	3.17	0.19	1.02	0.19	1.05	0.70	3.84
A. vaginae BV High Negative[3]	165[4]	16.56	0.00	0.00	0.25	1.53	0.18	1.11	0.56	3.38	0.13	0.79	0.12	0.70	0.67	4.02
A. vaginae BV Low Positive	168	15.11	0.00	0.00	0.19	1.25	0.15	0.97	0.51	3.40	0.12	0.82	0.12	0.78	0.59	3.92
A. vaginae BV Low Positive[2]	168	15.13	0.00	0.00	0.20	1.30	0.12	0.80	0.51	3.34	0.14	0.89	0.16	1.07	0.59	3.92
A. vaginae BV Mod Positive	168	14.13	0.08	0.54	0.21	1.50	0.17	1.21	0.51	3.63	0.08	0.57	0.20	1.40	0.62	4.41
A. vaginae BV Low Positive[2]	168	15.78	0.03	0.16	0.17	1.09	0.10	0.65	0.50	3.17	0.16	1.00	0.12	0.75	0.57	3.64
A. vaginae BV Low Positive[3]	168	15.61	0.00	0.00	0.23	1.47	0.15	0.94	0.51	3.29	0.10	0.66	0.18	1.15	0.62	3.95

CV = Coefficient of variation, Mod = moderate

[1] TTime is shown for *A. vaginae* only.

[2] Panel member contains 2 different organisms: results are shown for only the *A. vaginae* component.

[3] Panel member contains 3 different organisms: results are shown for only the *A. vaginae* component.

[4] Three invalid results were excluded from the analysis.

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 0.00.

Brief Description of Clinical Studies

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

Clinical Performance Study

This study was performed to demonstrate clinical performance characteristics for the Aptima BV assay. A multicenter study was conducted using prospectively-collected patient- and clinician-collected vaginal swab samples from women ≥14 years who were asymptomatic for or who exhibited signs and/or symptoms of vaginitis (ie, symptomatic). Twenty-one participating geographically diverse US private and academic family practice, obstetric-gynecologic, family planning, public health, STI, medical group clinics, and clinical research centers obtained vaginal

swab samples from 1519 symptomatic women, 174 asymptomatic women, and 4 women with unknown symptom status.

For each symptomatic subject, two vaginal swab samples (one patient-collected, one clinician-collected) were tested with the investigational Aptima BV assay. One clinician-collected vaginal swab sample was used for Nugent score evaluation, and modified Amsel criteria if necessary, to determine BV infection status. A Nugent interpretation established positive and negative BV reference status, except in cases of intermediate determinations. For intermediate Nugent interpretations, BV reference status was established using modified Amsel criteria. For each asymptomatic subject, one (1) clinician-collected vaginal swab sample was collected and tested with the investigational Aptima BV assay.

The clinical performance of the Aptima BV assay in symptomatic subjects (ie, the intended use population) was estimated relative to the BV infection status; sensitivity, specificity, PPV, and NPV were calculated for each Aptima sample type, along with corresponding 2-sided 95% CIs. Positivity rates were calculated for asymptomatic subjects.

Of the 1519 symptomatic subjects enrolled, 102 were not evaluable due to withdrawal (n = 17) or unknown BV infection status (n = 85). The remaining 1417 symptomatic subjects were evaluable for at least one of the sample types. Of the 174 asymptomatic subjects, 2 were not evaluable due to withdrawal; the remaining 172 asymptomatic subjects were evaluable. **Table 13** shows the demographic and baseline clinical characteristics of evaluable subjects.

Of the 1502 non-withdrawn symptomatic subjects, 1417 subjects were evaluable for the performance analyses for BV detection; results from 1405 patient-collected Aptima vaginal swab samples (99.2%, 1405/1417) and 1413 clinician-collected Aptima vaginal swab samples (99.7%, 1413/1417) were included in the performance analyses. All 172 non-withdrawn asymptomatic subjects were evaluable for the performance analyses for BV detection; results from the 172 clinician-collected Aptima vaginal swab samples were included in the positivity analysis.

Table 13: Summary of Demographics of Evaluable Subjects in the Aptima BV Assay Evaluation

	Symptomatic	Asymptomatic
Total, N	1417	172
Age, years		
Mean ± SD	34.7 ± 11.11	41.1 ± 13.22
Median	33.0	40.0
Range	14-75	18-73
Age category (years), n (%)		
14-17	4 (0.3)	0 (0.0)
18-29	537 (37.9)	42 (24.4)
30-39	469 (33.1)	42 (24.4)
40-49	235 (16.6)	36 (20.9)
>50	172 (12.1)	52 (30.2)
Race/Ethnicity, n (%)		
Asian	67 (4.7)	5 (2.9)
Black or African American	731 (51.6)	75 (43.6)
White (Hispanic or Latino)	248 (17.5)	41 (23.8)
White (Not Hispanic or Latino)	307 (21.7)	44 (25.6)
Other[1]	64 (4.5)	7 (4.1)
[1]Includes patient-reported other, mixed, and unknown races		

Performance characteristics for detection of BV infection for patient-collected and clinician-collected vaginal swab samples from symptomatic subjects were calculated overall (see **Table 14**), by race/ethnicity (see **Table 15**), and by clinical condition (see **Table 16**).

Table 14: Aptima BV Assay Performance Relative to BV Infection Status in Symptomatic Subjects

Specimen Type	N	TP	FP	TN	FN	Prevalence[1] (95% CI)[2]	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]	PPV (95% CI)[3]	NPV (95% CI)[3]
Patient-collected	1405	673	101[4]	612	19[5]	49.3	97.3 (95.8-98.2)	85.8 (83.1-88.2)	87.0 (84.8-88.9)	97.0 (95.5-98.1)
Clinician-collected	1413	660	75[6]	643	35[7]	49.2	95.0 (93.1-96.4)	89.6 (87.1-91.6)	89.8 (87.7-91.7)	94.8 (93.1-96.3)

FN = false negative, FP = false positive, NPV = negative predictive value, PPV = positive predictive value, TP = true positive, TN = true negative

[1]Study prevalence reported. [2]Score CI. [3]PPV 95% CI computed from the exact 95% CI for positive likelihood ratio; NPV 95% CI computed from the 95% for the negative likelihood ratio

[4] Of the 101 false positive results, 55 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 9 were positive by Amsel.

[5] Of the 19 false negative results, 6 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 7 were negative by Amsel..

[6] Of the 75 false positive results, 46 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 6 were positive by Amsel.

[7] Of the 35 false negative results, 10 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 15 were negative by Amsel.

Table 15: Aptima BV Assay Performance Relative to BV Infection Status in Symptomatic Subjects, by Race/Ethnicity

Race/Ethnicity	N	TP	FP	TN	FN	Prev[1] (%)	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]	PPV (95% CI)[3]	NPV (95% CI)[3]
Patient-collected										
Asian	65	19	6	39	1	30.8	95.0 (76.4-99.1)	86.7 (73.8-93.7)	76.0 (61.6-88.7)	97.5 (89.3-99.9)
Black / African American	727	434	43	239	11	61.2	97.5 (95.6-98.6)	84.8 (80.1-88.5)	91.0 (88.6-93.1)	95.6 (92.6-97.7)
White (Hispanic/Latino)	246	112	22	111	1	45.9	99.1 (95.2-99.8)	83.5 (76.2-88.8)	83.6 (78.0-88.6)	99.1 (95.6- 100)
White (Not Hispanic/Latino)	303	81	27	189	6	28.7	93.1 (85.8-96.8)	87.5 (82.4-91.3)	75.0 (68.1-81.5)	96.9 (94.0-98.8)
Other[4]	64	27	3	34	0	42.2	100 (87.5-100)	91.9 (78.7-97.2)	90.0 (76.9-97.7)	100 (91.3- 100)
Clinician-collected										
Asian	67	20	4	42	1	31.3	95.2 (77.3-99.2)	91.3 (79.7-96.6)	83.3 (68.2-94.3)	97.7 (89.8-99.9)
Black / African American	729	425	31	253	20	61.0	95.5 (93.2-97.1)	89.1 (84.9-92.2)	93.2 (90.9-95.2)	92.7 (89.4-95.2)
White (Hispanic/Latino)	247	110	18	115	4	46.2	96.5 (91.3-98.6)	86.5 (79.6-91.3)	85.9 (80.3-90.8)	96.6 (92.3-99.0)
White (Not Hispanic/Latino)	306	78	18	200	10	28.8	88.6 (80.3-93.7)	91.7 (87.3-94.7)	81.3 (73.9-87.5)	95.2 (92.1-97.5)
Other[4]	64	27	4	33	0	42.2	100 (87.5-100)	89.2 (75.3-95.7)	87.1 (74.2-96.0)	100 (91.1- 100)

FN = false negative, FP = false positive, NPV = negative predictive value, PPV = positive predictive value, Prev = prevalence TP = true positive, TN = true negative

[1]Study prevalence reported. [2]Score CI. [3]PPV 95% CI computed from the exact 95% CI for positive likelihood ratio; NPV 95% CI computed from the 95% for the negative likelihood ratio. [4]Includes patient-reported other, mixed, and unknown races.

Table 16: Aptima BV Assay Performance Relative to BV Infection Status in Symptomatic Subjects, by Clinical Condition

Clinical Condition	N	TP	FP	TN	FN	Prev[1] (%)	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]	PPV (95% CI)[3]	NPV (95% CI)[3]
Patient-collected										
Use of antibiotics	3	1	0	2	0	33.3	100 (20.7-100)	100 (34.2-100)	100 (7.8- 100)	100 (45.1- 100)
Use of antifungals	8	2	0	6	0	25.0	100 (34.2-100)	100 (61.0-100)	100 (33.3- 100)	100 (77.4- 100)
Use of estrogen therapy	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
Recurrent symptoms of vaginitis in the last 12 months	828	405	62	353	8	49.9	98.1 (96.2-99.0)	85.1 (81.3-88.2)	86.7 (83.9-89.3)	97.8 (95.9-99.0)
Unprotected intercourse in the last 24 hours	94	53	10	30	1	57.4	98.1 (90.2-99.7)	75.0 (59.8-85.8)	84.1 (76.5-91.0)	96.8 (85.6-99.9)
Pregnant	20	9	1	10	0	45.0	100 (70.1-100)	90.9 (62.3-98.4)	90.0 (66.5-99.7)	100 (77.9- 100)
With Menses	109	52	9	48	0	47.7	100 (93.1-100)	84.2 (72.6-91.5)	85.2 (76.6-92.4)	100 (93.6- 100)
Without Menses	1175	579	85	496	15	50.6	97.5 (95.9-98.5)	85.4 (82.3-88.0)	87.2 (84.9-89.3)	97.1 (95.4-98.3)
Post-menopausal	121	42	7	68	4	38.0	91.3 (79.7-96.6)	90.7 (82.0-95.4)	85.7 (75.5-93.2)	94.4 (88.1-98.3)
Clinician-collected										
Use of antibiotics	3	1	0	2	0	33.3	100 (20.7-100)	100 (34.2-100)	100 (7.8- 100)	100 (45.1- 100)
Use of antifungals	8	2	0	6	0	25.0	100 (34.2-100)	100 (61.0-100)	100 (33.3- 100)	100 (77.4- 100)
Use of estrogen therapy	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
Recurrent symptoms of vaginitis in the last 12 months	832	394	47	371	20	49.8	95.2 (92.7-96.9)	88.8 (85.4-91.4)	89.3 (86.6-91.8)	94.9 (92.5-96.7)
Unprotected intercourse in the last 24 hours	94	50	6	34	4	57.4	92.6 (82.4-97.1)	85.0 (70.9-92.9)	89.3 (81.2-95.4)	89.5 (78.4-96.6)
Pregnant	20	9	0	11	0	45.0	100 (70.1-100)	100 (74.1-100)	100 (74.2- 100)	100 (78.4- 100)
With Menses	111	50	8	51	2	46.8	96.2 (87.0-98.9)	86.4 (75.5-93.0)	86.2 (77.6-93.1)	96.2 (88.7-99.5)
Without Menses	1177	569	62	520	26	50.6	95.6 (93.7-97.0)	89.3 (86.6-91.6)	90.2 (88.0-92.2)	95.2 (93.3-96.8)
Post-menopausal	125	41	5	72	7	38.4	85.4 (72.8-92.8)	93.5 (85.7-97.2)	89.1 (79.1-95.8)	91.1 (84.7-95.9)

FN = false negative, FP = false positive, NC = not calculable, NPV = negative predictive value, PPV = positive predictive value, Prev = prevalence TP = true positive, TN = true negative

[1]Study prevalence reported. [2]Score CI. [3]PPV 95% CI computed from the exact 95% CI for positive likelihood ratio; NPV 95% CI computed from the 95% for the negative likelihood ratio.

Positivity rates were estimated for clinician-collected vaginal swab samples from asymptomatic subjects overall and by race/ethnicity (see **Table 17**).

Table 17: Positivity of BV Infection as Determined by the Aptima BV Assay in Asymptomatic Subjects

	% Positivity (# positive/# tested with valid results)
All	40.7% (70/172)
Asian	40.0% (2/5)
Black/African American	52.0% (39/75)
White (Hispanic/Latino)	43.9% (18/41)
White (Not Hispanic/Latino)	15.9% (7/44)
Other	57.1% (4/7)

Reproducibility

Aptima BV assay reproducibility was evaluated at three US sites using seven panel members. Testing was performed using one lot of assay reagents and six operators (two at each site). At each site, testing was performed for at least six days. Each run had three replicates of each panel member.

The panel members were made using a simulated vaginal swab matrix ('SVSM', which contains specimen transport media (STM) spiked with simulated vaginal fluid) negative for *Lactobacillus* species, *G. vaginalis*, and *A. vaginae*. Six panel members contained cell lysates of at least 1 of the following organisms: *L. crispatus*, *L. jensenii*, *G. vaginalis*, or *A. vaginae*; different bacterial combinations were prepared to represent the variety of targeted BV organism combinations present in vaginal specimens. One negative panel member contained only the matrix with no added target analytes.

The agreement with expected results was 100% for all panel members containing *L. crispatus*, *L. jensenii*, *G. vaginalis*, or *A. vaginae*, as shown in **Table 18**.

Table 18: Agreement of Aptima BV Assay Results With Expected Results

Panel Description	Expected Results	N	Agreement, % (95% CI)
True Neg	Negative	36/36	100 (96.6-100)
BV Neg[1]	Negative	36/36	100 (96.6-100)
Gvag Low Pos	Positive	36/36	100 (96.6-100)
Avag Low Pos	Positive	36/36	100 (96.6-100)
BV Low Pos[1]	Positive	36/36	100 (96.6-100)
Gvag Mod Pos	Positive	36/36	100 (96.6-100)
Avag MosPos	Positive	36/36	100 (96.6-100)

Avag = *A. vaginae*, CI = Score confidence interval, Gvag = *G. vaginalis*, Mod=moderate, Neg = negative, Pos = positive
[1] Panel member contains 2 different organisms.

Across organisms/panel members, the total %CV values ranged from 4.21% to 4.76%; total SD values were ≤1.12. For most panel members, the "between sites," "between operators," and "between runs" factors were the largest contributors to total variability; for all other sources of variation, SD values were ≤0.18 (%CV values were ≤1.13%), as shown in **Table 19**.

Table 19: Signal Variability of the Aptima BV Assay by Analyte-positive Panel Member

			Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Panel Description	N	Mean TTime[1]	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Lcrisp BV Neg[2]	108	19.73	0.30	1.53	0.61	3.07	0.13	0.64	0.63	3.17	0.12	0.62	0.94	4.76
Ljen Low Pos[2]	108	24.31	0.00	0.00	0.77	3.16	0.00	0.00	0.80	3.28	0.15	0.62	1.12	4.60
Gvag Low Pos	108	15.69	0.35	2.26	0.40	2.52	0.00	0.00	0.38	2.43	0.15	0.96	0.67	4.28
Gvag Mod Pos	108	14.33	0.30	2.07	0.37	2.58	0.00	0.00	0.35	2.41	0.14	0.98	0.60	4.21
Avag BV Neg[2]	108	18.01	0.39	2.15	0.44	2.46	0.08	0.45	0.47	2.59	0.18	0.97	0.78	4.30
Avag Low Pos	108	14.95	0.38	2.52	0.41	2.75	0.00	0.00	0.39	2.61	0.14	0.93	0.69	4.64
Avag Low Pos[2]	108	14.94	0.41	2.76	0.37	2.51	0.00	0.00	0.37	2.45	0.17	1.13	0.69	4.60
Avag Mod Pos	108	13.99	0.29	2.08	0.36	2.60	0.03	0.18	0.39	2.82	0.14	1.00	0.63	4.48

Avag = *A. vaginae*, CV = coefficient of variation, Gvag = *G. vaginalis*, Lcrisp = *L. crispatus*, Ljen = *L. jensenii*, Mod = moderate, Neg = negative, Pos = positive, SD = standard deviation, TTime = emergence time of a signal (above a specific threshold)

Note: If variability from a factor was numerically negative, SD and CV are shown as 0.0.

[1] The assay reports TTime for each assay analyte separately; the mean and signal variability reported are for the TTime corresponding to the analyte(s) present in each panel member.

[2] Panel member contains 2 different organisms; results are shown for only the component shown.

For Aptima BV assay controls and positive calibrators, the total %CV values ranged from 4.47% to 5.36%; total SD values were ≤1.11 (see **Tables 20** and **21**, respectively).

Table 20: Signal Variability of the Aptima BV Assay Positive Controls

			Between Sites		Between Operators		Between Days		Within Runs		Total	
Control	N	Mean Ct	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Neg - Lcrisp	36	20.34	0.24	1.16	0.74	3.63	0.00	0.00	0.71	3.48	1.05	5.16
Pos - Gvag	36	17.52	0.05	0.28	0.60	3.41	0.00	0.00	0.52	2.96	0.79	4.52
Pos - Avag	36	13.79	0.27	1.97	0.38	2.77	0.00	0.00	0.41	2.97	0.62	4.51

Avag = *A. vaginae*, CV = coefficient of variation, Gvag = *G. vaginalis*, Lcrisp = *L. crispatus*, Ljen = *L. jensenii*, Mod = moderate, Neg = negative, Pos = positive, SD = standard deviation, TTime = emergence time of a signal (above a specific threshold)

Table 21: Signal Variability of the Aptima BV Assay Positive Calibrators

			Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Analyte	N	Mean TTime	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Lcrisp	108	20.75	0.00	0.00	0.78	3.77	0.00	0.00	0.78	3.75	0.13	0.64	1.11	5.36
Gvag	108	19.03	0.34	1.78	0.61	3.19	0.00	0.00	0.56	2.96	0.15	0.79	0.91	4.76
Avag	108	18.46	0.41	2.23	0.48	2.62	0.08	0.42	0.51	2.76	0.11	0.58	0.83	4.47

Avag = *A. vaginae*, CV = coefficient of variation, Gvag = *G. vaginalis*, Lcrisp = *L. crispatus*, SD = standard deviation, TTime = emergence time of a signal (above a specific threshold)

VIII. CONCLUSIONS

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