510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K190472

B. Purpose for Submission:

New device 510k clearance for the Aptima CV/TV assay performed on the Panther System

C. Measurand:

RNA component of RNAse P ribonucleoprotein of *Candida glabrata* and the *Candida* species group (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*) (C spp); ribosomal RNA (rRNA) of *Trichomonas vaginalis* (TV)

D. Type of Test:

Qualitative Nucleic Acid Amplification - Real Time Transcription-Mediated Amplification (TMA)

E. Applicant:

Hologic, Inc.

F. Proprietary and Established Names:

Aptima CV/TV Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3975
   Device that detects nucleic acid sequences from microorganisms associated with vaginitis and bacterial vaginosis

2. Classification:

   Class II

3. Product code:

   PQA - Vaginitis and bacterial vaginosis nucleic acid detection system
   NSU - Instrumentation for clinical multiplex test systems

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Aptima CV/TV assay is an *in vitro* nucleic acid amplification test for the detection of RNA from microorganisms associated with vulvovaginal candidiasis and trichomoniasis. The assay utilizes real time transcription-mediated amplification (TMA) to detect and qualitatively report results for the following organisms:

   - *Candida* species group (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*)
   - *Candida glabrata*
   - *Trichomonas vaginalis*

   The assay differentiates between *Candida glabrata* and the *Candida* species group (C spp) by targeting the RNA component of RNAse P ribonucleoprotein; the assay does not differentiate among C spp. For *Trichomonas vaginalis*, the assay targets ribosomal RNA (rRNA) and differentiates the result from results for *Candida glabrata* and C spp. The assay is intended to aid in the diagnosis of vulvovaginal candidiasis and trichomoniasis on the automated Panther system using clinician-collected and patient-collected vaginal swab specimens from females with a clinical presentation consistent with vaginitis or vulvovaginitis.

2. Indication(s) for use:

   Same as Intended Use(s)

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Panther System

I. Device Description:

The Aptima CV/TV assay is an *in vitro* nucleic acid amplification test for the detection and

quantitation of RNA from microorganisms associated with vulvovaginal candidiasis and trichomoniasis, in women with a clinical presentation consistent with vaginitis and vulvovaginitis. The Aptima CV/TV assay utilizes the automated Panther system to provide qualitative results to aid in the diagnosis of vulvovaginal candidiasis and trichomoniasis.

Reagents and Materials Provided

The Aptima CV/TV assay is provided as a 100-test kit. The Aptima CV/TV assay master kit contains eight reagents, one calibrator, and two controls required for sample processing. There are four boxes that make up the assay master kit. Boxes 1 and 2 contain the Aptima CV/TV 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 CV/TV 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 CV/TV assay are detailed in Table 1 below.

Table 1: Reagents Required to Perform the Aptima CV/TV Assay (Test Kit)

Box	**Symbol**	**Component**	**Quantity**
1	A	Amplification Reagent (Non-infectious nucleic acids dried in buffered soliution.)	1 vial
	E	Enzyme Reagent (Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.)	1 vial
	PRO	Promotor Reagent (Non-infectious nucleic acids dried in buffered soliution.)	1 vial
	IC	Internal Control (Non-infectious nucleic acids in buffered soliution.)	1 x 0.3 mL
2	AR	Amplification Reconstitution Solution (Aqueous solution containing glycerol and preservatives.)	1 x 7.2 mL
	ER	Enzyme Reconstitution Solution (HEPES buffered solution containing a surfactant and glycerol.)	1 x 5.8 mL
	PROR	Promotor Reconstitution Solution (Aqueous solution containing glycerol and preservatives.)	1 x 4.5 mL
	TCR	Target Capture Reagent (Buffered salt solution containing non-infectious nucleic acids and magnetic particles.)	1 x 26.0 mL
		Reconstitution Collars	3
		Master Lot Barcode Sheet	1 sheet
3	PCAL	Positive Calibrator (Non-infectious nucleic acids in buffered soliution.)	5 x 2.8 mL
		Calibrator Barcode Label	1 sheet
4	CONTROL-	Negative Control (Buffered solution.)	5 x 2.7 mL
	CONTROL+	Positive Control (Non-infectious *C. albicans*, *C. glabrata*, and *T. vaginalis* cultured organisms in buffered solution.)	5 x 1.7 mL
		Control Barcode Label	1 sheet

Instrumentation

The Aptima CV/TV 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 CV/TV 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.

Materials Required but Available Separately

A listing of the materials that are required to perform the Aptima CV/TV assay on the Panther system but available separately from the Aptima CV/TV assay test kit are detailed in Table 2 below.

Table 2: Materials Required to Perform the Aptima CV/TV Assay but Available Seperately

Maretial
Aptima Multitest Swab Specimen Collection Kit
Panther Run Kit for Real Time Assays (for real time assays only) • Aptima Assay Fluids Kit (also known as Universal Fluids Kit). Contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent • Multi-tube units (MTUs) • Panther Waste Bag Kit • Panther Waste Bin Cover
Or, Panther System Run Kit, when running non-real time-TMA assays in parallel with real time-TMA assays Contains MTUs, waste bags, waste bin covers, auto detect, and assay fluids
Aptima Assay Fluids Kit Contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent
Multi-tube units (MTUs)
Tips, 1000 µL conductive, liquid sensing
Aptima penetrable caps
Replacement non-penetrable caps
Reagent Replacement Caps (for Amplification, Enzyme, and Promoter reagent reconstitution bottles, and TCR bottle)
Tube Rocker
Plastic-backed laboratory bench covers
Lint-free wipes
Pipettor and tips
Bleach, 5.0% to 7.0% (0.7 M to 1.0 M) sodium hypochlorite solution
Disposable, powderless gloves

Quality Control

Assay Calibration

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

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

Negative and Positive Controls

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

During processing, criteria for acceptance of controls are automatically verified by software on the Panther system. If any one of the controls has an invalid result, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Internal Control (IC)

Each sample contains an IC. During processing, IC acceptance criteria are automatically verified by the Panther system software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested to obtain a valid result. The Panther system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the Panther System Operator's Manual.

Sample and Assay Run Validity

The Panther software assesses the validity of all samples, calibrators and controls. Only the results of specimens that are valid will be reported to the user.

In the Aptima CV/TV assay, only the C spp channel is subjected to in-run calibration. If the reagents have valid calibration and controls, the Panther system calculates the specimen results automatically for the user.

Sample Validity is determined from several factors:

- Samples are invalidated if the estimated background relative fluorescence units (RFU) signal exceeds a predefined background threshold.
- For samples determined to be positive for one or more analytes (C spp, *C.glabrata* or *T. vaginalis*), the IC signal is not utilized in determining sample validity.
- For samples determined to be negative in all three analyte channels, the sample IC must be valid for the negative result to be valid. To be valid, the IC amplification curve time of emergence (TTime), emergence rate, and relative fluorescence units (RFU) must be within specified ranges. If the IC is not within these ranges, the sample is invalidated by the Panther software.

Run Validity is determined by the calibrator and control performance:

- Calibration is evaluated on the FAM (C spp) channel only. Replicates of the calibrator must meet RFU range, TTime, and emergence rate criteria to be valid. Outlier analysis from the three replicates of calibrator may be triggered by predefined

%CV criteria. Calibrator replicates remaining after outlier removal are used along with the calibration coefficient found on the Master Lot Barcode Sheet to generate a calibration curve. Each calibrator replicate is quantitated and must fall within an acceptable deviation for the calibration curve to be valid. A failing replicate may be removed and the curve regenerated as long as two valid replicates remain. If there are less than two valid calibrator replicates, the calibration fails and the run is invalid.

- The CV/TV negative control must meet amplification criteria (RFU range, TTime, and emergence rate) for the Cy5.5 (IC) channel, and no amplification on the FAM (C spp), HEX (*C.glabrata*), or ROX (*T. vaginalis*) channels.
- The CV/TV positive control must report POS for C spp, *C.glabrata*, and *T. vaginalis* analytes, i.e., it must meet amplification criteria (RFU range, TTime, and emergence rate) for the FAM, the HEX, and the ROX channels. In addition, the calculated log copy value for C spp must fall within a specified range.

An operator may also manually invalidate an individual specimen or an entire run if it was observed and documented that a procedural, technical, or instrument-related error occurred while performing the assay.

Results Generation

Assay results are automatically generated by the Panther system Aptima CV/TV assay software.

A qualitative result is separately reported for C spp (positive or negative). Calibration of the C spp channel is used to determine if the C spp concentration is above or below the C spp positivity assay cutoff.

Qualitative results are also separately reported for *C. glabrata* and *T. vaginalis*. Calibration is not utilized for these analytes. To determine positivity, minimum specifications for amplification based on TTime, emergence rate, and RFU range must be met.

When a sample is co-infected with C spp and a high concentration of *T. vaginalis* (as determined by *T. vaginalis* TTime), C spp positivity is determined by the detection of C spp, regardless of C spp concentration as determined by calibration. In this scenario, C spp positivity must meet minimum specifications for amplification based on TTime, emergence rate, and RFU range.

Test results may also be masked by the user (No Test); the user may pre-select to not report (mask) one or more analyte results.

Results Interpretation

The user interprets the results generated by the Panther system based on Table 3 below.

Table 3: Aptima CV/TV Assay Result Interpretation

C spp Result	***C. glabrata* Result**	***T. vaginalis* Result**	**Results Valid or Invalid**	**Interpretation**
Positive	Negative	Negative	Valid	*Candida* species group RNA detected; *Candida glabrata* RNA and *Trichomanas vaginalis* RNA not detected.
Positive	Positive	Negative	Valid	*Candida* species group RNA and *Candida glabrata* RNA detected; *Trichomanas vaginalis* RNA not detected.
Positive	Negative	Positive	Valid	*Candida* species group RNA and *Trichomanas vaginalis* RNA detected; *Candida glabrata* RNA not detected.
Positive	Positive	Positive	Valid	*Candida* species group RNA, *Candida glabrata* RNA, and *Trichomanas vaginalis* RNA detected.
Negative	Positive	Negative	Valid	*Candida glabrata* RNA detected; *Candida* species group RNA and *Trichomanas vaginalis* RNA not detected.
Negative	Negative	Positive	Valid	*Trichomanas vaginalis* RNA detected; *Candida* species group RNA and *Candida glabrata* RNA not detected.
Negative	Positive	Positive	Valid	*Candida glabrata* RNA and *Trichomanas vaginalis* RNA detected; *Candida* species group RNA not detected.
Negative	Negative	Negative	Valid	*Candida* species group RNA, *Candida glabrata* RNA, and *Trichomonas vaginalis* RNA not detected.
Invalid	Invalid	Invalid	Invalid	Invalid: there was an error in the generation of the result. Specimen should be retested.

Note: *Candida* species group = *C. albicans, C. parapsilosis, C. dubliniensis,* and/or *C. tropicalis*

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BD MAX Vaginal Panel

2. Predicate 510(k) number(s):

   DEN160001

3. Comparison with predicate:

Similarities and Differences		
Item	**Device**	**Predicate**
	Aptima CV/TV Assay (on the Pather System) K190472	**BD MAX Vaginal Panel (on the BD MAX System) DEN160001**
Intended Use	The Aptima CV/TV assay is an in vitro nucleic acid amplification test for the detection of RNA from microorganisms associated with vulvovaginal candidiasis and trichomoniasis. The assay utilizes real time transcription-mediated amplification (TMA) to detect and qualitatively report results for the following organisms: • *Candida* species group (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*)	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

Similarities and Differences		
Item	**Device**	**Predicate**
	Aptima CV/TV Assay (on the Pather System) K190472	**BD MAX Vaginal Panel (on the BD MAX System) DEN160001**
	• *Candida glabrata* • *Trichomonas vaginalis* The assay differentiates between *Candida glabrata* and the *Candida* species group (C spp) by targeting the RNA component of RNAse P ribonucleoprotein; the assay does not differentiate among C spp. For *Trichomonas vaginalis*, the assay targets ribosomal RNA (rRNA) and differentiates the result from results for *Candida glabrata* and C spp. The assay is intended to aid in the diagnosis of vulvovaginal candidiasis and trichomoniasis on the automated Panther system using clinician-collected and patient-collected vaginal swab specimens from females with a clinical presentation consistent with vaginitis or vulvovaginitis.	targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA from • Bacterial vaginosis markers (Individual markers not reported) o *Lactobacillus spp*. (*L. crispatus* and *L. jensenii*) o *Gardnerella vaginalis* o *Atopobium vaginae* o Bacterial Vaginosis Associated Bacteria-2 (BVAB-2) o *Megasphaera*-1 • *Candida* spp. (*C. albicans, C. 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.
Sample Type	Vaginal swabs in female patients who are symptomatic for vaginitis or vulvovaginitis	Same
Patient Population	Women with a clinical presentation consistent with vaginitis or vulvovaginitis	Same
Organisms Detected	*Candida* species (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*); *Candida glabrata*; *Trichomonas vaginalis*	*Lactobacillus* (*L. crispatus*, and *L. jensenii*), *Gardnerella vaginalis*, *Atopobium vaginae*, Bacterial Vaginosis Associated Bacteria-2 (BVAB-2), *Megasphaera*-1, *Candida* (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*), *Candida glabrata*, *Candida krusei*, *Trichomonas vaginalis*
Platform/Technology Principle of Operation	Panther System/Real-time Transcription Mediated Amplification (TMA)	BD MAX System/Real-time polymerase chain reaction (PCR)
Analyte	RNA	DNA
Assay Controls	Incorporates an Internal Control in every test. Uses external positive and negative controls.	Same
Assay Calibrators	Positive Calibrator for the FAM (C spp) channel	None

K. Standard/Guidance Document Referenced (if applicable):

None

L. Test Principle:

The Aptima CV/TV 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 organisms, releases the RNA, and protects it from degradation during storage. When the 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 C spp, *C. glabrata*, TV, and IC amplification products. The Panther system software uses an Aptima CV/TV assay-specific algorithm to generate a Positive or Negative status for each target organism in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

      Within Laboratory Precision Study

      Within Laboratory Precision was evaluated on three Panther systems at one testing site. Three operators performed testing across 22 days and three reagent lots. Each operator performed two runs per day using a seven-member sample panel. Each run consisted of three replicates of each panel member. The panel members were prepared with *C. albicans*, *C. glabrata* or *T. vaginalis* in simulated vaginal swab matrix (SVSM). The six positive panel members targeted *C. albicans* at Low (approximately 2x C_{95}) and Moderate (3x C_{95}) Positive levels, *C. glabrata* at Low (approximately 2x

LoD) and Moderate (3x LoD) Positive levels, and *T. vaginalis* at Low (approximately 2x LoD) and Moderate (3x LoD) Positive levels. One Negative panel member contained SVSM with no added target analytes.

The study results are presented in Table 4 below. Signal variability of the Aptima CV/TV assay in terms of amplification curve time of emergence (TTime) was also calculated for analyte positive panel members. Variability calculated between instruments, between operators, between lots, between days, between runs, within runs, and overall, is shown in Table 5 below.

Table 4: Within Laboratory Precision - Agreement of Aptima CV/TV Assay with Expected Results

Panel Member (Analyte Composition)	**Positive/Total N**	**Expected Positivity**	**Percent Positivity (95% CI)**
Negative (SVSM)	0/162	0%	0 (0.0-2.3)
Low Positive (*C. albicans*)	162/162	≥ 95%	100 (97.7-100.0)
Low Positive (*C. glabrata*)	162/162	≥ 95%	100 (97.7-100.0)
Low Positive (*T. vaginalis*)	162/162	≥ 95%	100 (97.7-100.0)
Moderate Positive (*C. albicans*)	162/162	100%	100 (97.7-100.0)
Moderate Positive (*C. glabrata*)	162/162	100%	100 (97.7-100.0)
Moderate Positive (*T. vaginalis*)	162/162	100%	100 (97.7-100.0)

Table 5: Within Laboratory Precision - Signal Varability of the Aptima CV/TV Assay by Analyte Positive Panel Member

			Between Days		**Between Instruments**		**Between Operators**		**Between Lots**		**Between Runs**		**With Runs**		**Total**	
Panel Member (Analyte Composition)	**N**	**Mean TTime[1]**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Low Positive (*C. albicans*)	162	14.96	0.12	0.82	0.00	0.00	0.24	1.59	0.54	3.58	0.23	1.52	0.28	1.84	0.70	4.66
Low Positive (*C. glabrata*)	162	21.07	0.00	0.00	0.15	0.69	0.25	1.18	0.14	0.65	0.19	0.89	0.40	1.91	0.55	2.59
Low Positive (*T. vaginalis*)	162	24.09	0.00	0.00	0.33	1.38	0.22	0.93	0.01	0.05	0.21	0.87	0.59	2.46	0.75	3.09
Moderate Positive (*C. albicans*)	162	14.62	0.11	0.72	0.00	0.00	0.22	1.47	0.43	2.95	0.26	1.77	0.24	1.62	0.60	4.14
Moderate Positive (*C. glabrata*)	162	20.63	0.00	0.00	0.00	0.00	0.26	1.27	0.31	1.50	0.26	1.25	0.52	2.51	0.71	3.42
Moderate Positive (*T. vaginalis*)	162	22.73	0.00	0.00	0.12	0.54	0.24	1.08	0.18	0.80	0.28	1.23	0.41	1.79	0.59	2.61

CV = coefficient of variation, SD = standard deviation, TTime = amplification curve time of emergence (above a specific threshold)
Note: If variability from a factor was numerically negative, SD and CV are shown as 0.00.
[1] The assay reports TTime for each assay analyte separately; the mean and signal variability reported are for the TTime corresponding to the analyte present in each panel member.

Multi-Site Reproducibility

Aptima CV/TV assay reproducibility was evaluated at three US sites testing a seven-member sample panel. 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 operator performed one run per day, and each run had three replicates of each panel member. This study design allowed the generation of 108 data points for each panel member (i.e., 3 replicates/run/operator x 1 run/day x 2 operators/site x 6 days x 3

sites = 108).

The panel members were prepared using a simulated vaginal swab matrix (SVSM) negative for *Candida* species (including *C. glabrata*) and *T. vaginalis*. Six panel members contained cell lysates of one of the following organisms: *C. albicans* (used as a representative of the *Candida* species group), *C. glabrata*, or *T. vaginalis*. Low Positive and Moderate Positive concentrations of each analyte were tested. One negative panel member contained only the SVSM with no added target analytes. The six positive panel members targeted *C. albicans* at Low (approximately 2x C_{95}) and Moderate (3x C_{95}) Positive, *C. glabrata* at Low (approximately 2x LoD) and Moderate (3x LoD) Positive, and *T. vaginalis* at Low (approximately 2x LoD) and Moderate (3x LoD) Positive.

While the study design allowed repeat testing of invalid runs in accordance with the Aptima CV/TV Assay Instructions for Use using a new set of assay calibrator, controls, and samples, tested samples with invalid results and samples with "error" results in a valid run were not retested in this study. Samples with invalid and "error" results were excluded from the performance analyses.

A total of 36 runs were performed during this study. Of the 36 runs, all 36 (100%, 95% CI: 90.4% - 100%) were valid. Of the 756 samples tested in valid runs, 751 samples (99.3%, 95% CI: 98.5% - 99.7%) had valid Aptima CV/TV assay results, and 5 samples (0.7%, 95% CI: 0.3% - 1.5%) had invalid results due to hardware error and were excluded from the performance analyses.

The reproducibility study results are presented in Table 6 and Table 7 below. Signal variability of the Aptima CV/TV assay in terms of amplification curve time of emergence (TTime) was also calculated for analyte positive panel members. Variability calculated between sites, between operators, between days, between runs, within runs, and overall, is shown in Table 8 below.

Table 6: Multi-Site Reproducibility Study - Agreement of Aptima CV/TV Assay Results With Expected Results on the Panther System by Site

		Site 1		Site 2		Site 3	
Target	**Concentration**	**Agreed N/ Total N**	**Agreement (%) (95% CI)**	**Agreed N/ Total N**	**Agreement (%) (95% CI)**	**Agreed N/ Total N**	**Agreement (%) (95% CI)**
C. albicans	Negative	180/180	100 (97.9-100)	180/180	100 (97.9-100)	176/176	100 (97.9-100)
	Low Positive (approximately 2x C_{95})	36/36	100 (90.4-100)	36/36	100 (90.4-100)	36/36	100 (90.4-100)
	Moderate Positive (3x C_{95})	36/36	100 (90.4-100)	36/36	100 (90.4-100)	35/35	100 (90.1-100)
C. glabrata	Negative	180/180	100 (97.9-100)	180/180	100 (97.9-100)	178/178	100 (97.9-100)
	Low Positive (approximately 2x LoD)	36/36	100 (90.4-100)	36/36	100 (90.4-100)	34/34	100 (89.8-100)
	Moderate Positive (3x LoD)	36/36	100 (90.4-100)	36/36	100 (90.4-100)	35/35	100 (90.1-100)
T. vaginalis	Negative	180/180	100 (97.9-100)	180/180	100 (97.9-100)	176/176	100 (97.9-100)
	Low Positive (approximately 2x LoD)	36/36	100 (90.4-100)	36/36	100 (90.4-100)	36/36	100 (90.4-100)
	Moderate Positive (3x LoD)	36/36	100 (90.4-100)	36/36	100 (90.4-100)	35/35	100 (90.1-100)

Table 7: Multi-Site Reproducibility Study - Agreement of Aptima CV/TV Assay Results With Expected Results on the Panther System by Panel Member

Target	**Concentration**	**Agreed N/Total N**	**Agreement (%) (95% CI)**
C. albicans	Negative [1]	536/536	100 (99.3-100)
	Low Positive (approximately 2x C_{95})	108/108	100 (96.6-100)
	Moderate Positive (3x C_{95})	107/107	100 (96.5-100)
C. glabrata	Negative [1]	538/538	100 (99.3-100)
	Low Positive (approximately 2x LoD)	106/106	100 (96.5-100)
	Moderate Positive (3x LoD)	107/107	100 (96.5-100)
T. vaginalis	Negative [1]	536/536	100 (99.3-100)
	Low Positive (approximately 2x LoD)	108/108	100 (96.6-100)
	Moderate Positive (3x LoD)	107/107	100 (96.5-100)

[1] Includes all panel members without the target present.

Table 8: Multi-Site Reproducibility - Signal Varability of the Aptima CV/TV Assay by Analyte Positive Panel Member

			Between Sites		Between Operators		Between Days		Between Runs		With Runs		Total	
Panel Member (Analyte Composition)	**N**	**Mean TTime[1]**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Low Positive (*C. albicans*)	108	14.68	0.66	4.47	0.00	0.00	0.00	0.00	0.00	0.00	0.30	2.02	0.83	5.64
Moderate Positive (*C. albicans*)	107	14.37	0.66	4.58	0.14	0.99	0.00	0.00	0.00	0.00	0.28	1.98	0.81	5.64
Low Positive (*C. glabrata*)	106	21.36	0.84	3.94	0.18	0.84	0.00	0.00	0.00	0.00	0.62	2.89	1.26	5.88
Moderate Positive (*C. glabrata*)	107	20.54	0.99	4.83	0.30	1.46	0.00	0.00	0.00	0.00	0.48	2.34	1.37	6.68
Low Positive (*T. vaginalis*)	108	24.32	1.16	4.77	0.00	0.00	0.00	0.00	0.00	0.00	0.60	2.48	1.59	6.54
Moderate Positive (*T. vaginalis*)	107	23.09	1.18	5.13	0.00	0.00	0.00	0.00	0.00	0.00	0.56	2.41	1.56	6.77

CV = coefficient of variation, SD = standard deviation, TTime = amplification curve time of emergence (above a specific threshold)

Note: If variability from a factor was numerically negative, SD and CV are shown as 0.00.

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

b. Linearity/assay reportable range:

Although a final qualitative result is reported for C spp (positive or negative), calibration of the C spp (FAM) channel is initially used to generate a quantitative concentration value in log copies/mL, and then a determination is made whether the C spp concentration in log copies/mL is above or below the C spp positivity assay cutoff.

The linearity of the four *Candida* species detected in the C spp (FAM) channel, *C. albicans, C. parapsilosis, C. dubliniensis,* and *C. tropicalis,* was assessed by testing serial dilutions of lysate in SVSM for each of the four *Candida* species, and performing a polynomial regression analysis to determine an acceptable degree of non-linearity for each *Candida* species detected in the C spp (FAM) channel. The serial dilutions spanned the clinically relevant range of the assay. Testing was performed on one Panther system using one reagent lot.

For each of the four *Candida* species detected in the C spp (FAM) channel, six serial dilutions in total were prepared to show a larger dynamic range of linearity for each organism, and concentrations were adjusted to maintain 100% positivity for all dilutions. Cell lysate stocks with a confirmed CFU/mL concentration for each organism were used to build the serial dilutions at the concentrations listed in Table 9 below:

Table 9: Linearity Study Dilutions per *Candida* Species

Dilution Concentrations (CFU/mL)							
Dilution #	***Candida albicans***	**Dilution #**	***Candida parapsilosis***	**Dilution #**	***Candida dubliniensis***	**Dilution #**	***Candida tropicalis***
1	3.0×10^5	1	3.0×10^5	1	1.0×10^5	1	1.0×10^5
2	1.0×10^5	2	1.0×10^5	2	3.0×10^4	2	3.0×10^4
3	3.0×10^4	3	3.0×10^4	3	1.0×10^4	3	1.0×10^4
4	1.0×10^4	4	1.0×10^4	4	3.0×10^3	4	3.0×10^3
5	3.0×10^3	5	3.0×10^3	5	1.0×10^3	5	1.0×10^3
6	2.0×10^3	6	1.0×10^3	6	3.0×10^2	6	7.5×10^2

Five replicates per dilution were tested. Dilutions were randomized within run. Only valid data was used in data analysis.

Determination of linearity was performed by polynomial regression analysis per CLSI Guideline EP6-A2. The acceptance criteria for the linearity study were 1) degree of non-linearity should not exceed the ± 0.50 log copies/mL range at all concentrations tested; and 2) standard deviation of the observed log copies/mL value should be ≤ 0.25 at all concentrations tested to meet the repeatability requirements.The linearity study results for *C. albicans* are presented in Table 10 to Table 12 below.

Table 10: Degree of Non-Linearity for *C. albicans*

Best Fit Polynomial	**Dilution #**	**N**	**Linear Fit Calculated Mean (Log copies/mL)**	**Best Fit Polynomial Calculated Mean (Log copies/mL)**	**Non-Linearity Best Fit Polynomial Caculated Mean – Linear Fit Calculated Mean (Log copies/mL)**	**Degree of Non-Linearity is within ± 0.5 Log copies/mL (Yes or No)**
3rd Degree Polynomial	1	5	4.819	4.811	-0.008	Yes
	2	5	4.297	4.225	-0.072	Yes
	3	5	3.774	3.810	0.036	Yes
	4	5	3.252	3.383	0.130	Yes
	5	5	2.730	2.756	0.026	Yes
	6	5	2.521	2.409	-0.113	Yes

Table 11: Repeatability for *C. albicans*

***Candida* Species**	**Dilution #**	**N**	**Observed Mean (Log copies/mL)**	**Observed Standatd Deviation (Log copies/mL)**	**Standard Deviation ≤ 0.25 Log copies/mL (Yes or No)**
C. albicans	1	5	4.799	0.081	Yes
	2	5	4.267	0.103	Yes
	3	5	3.757	0.090	Yes
	4	5	3.401	0.138	Yes
	5	5	2.777	0.191	Yes
	6	5	2.391	0.176	Yes

Table 12: *C. albicans* – Deviations between the Observed Mean and the Linear Fit Calculated Mean

***Candida* Species**	**Dilution #**	**N**	**Observed Mean (Log copies/mL)**	**Observed Standatd Deviation (Log copies/mL)**	**Linear Fit Calculated Mean (Log copies/mL)**	**Deviation Observed Mean - Best Fit Polynomial Calculated Mean (Log copies/mL)**
C. albicans	1	5	4.799	0.081	4.819	-0.020
	2	5	4.267	0.103	4.297	-0.030
	3	5	3.757	0.090	3.774	-0.017
	4	5	3.401	0.138	3.252	0.149
	5	5	2.777	0.191	2.730	0.047
	6	5	2.391	0.176	2.521	-0.130

Note: The bolded value indicates the maximum deviation between the Observed Mean in log copies/mL and the Linear Fit Calculated Mean in log copies/mL.

C. parapsilosis did not have a statistically significant 2nd or 3rd degree polynomial model fit, therefore, the linear fit was determined to be the best fit for this species. The linearity study results for *C. parapsilosis* are presented in Table 13 and Table 14 below.

Table 13: Repeatability for *C. parapsilosis*

***Candida* Species**	**Dilution #**	**N**	**Observed Mean (Log copies/mL)**	**Observed Standatd Deviation (Log copies/mL)**	**Standard Deviation ≤ 0.25 Log copies/mL (Yes or No)**
C. parapsilosis	1	5	4.214	0.208	Yes
	2	5	3.834	0.037	Yes
	3	5	3.280	0.154	Yes
	4	5	2.806	0.186	Yes
	5	5	2.301	0.117	Yes
	6	5	1.819	0.108	Yes

Table 15: *C. parapsilosis* –Deviations between the Observed Mean and the Linear Fit Calculated Mean

***Candida* Species**	**Dilution #**	**N**	**Observed Mean (Log copies/mL)**	**Observed Standatd Deviation (Log copies/mL)**	**Linear Fit Calculated Mean (Log copies/mL)**	**Deviation (Observed Mean - Linear Fit Calculated Mean) (Log copies/mL)**
C. parapsilosis	1	5	4.214	0.208	4.260	-0.046
	2	5	3.834	0.037	3.773	**0.061**
	3	5	3.280	0.154	3.286	-0.006
	4	5	2.806	0.186	2.799	0.007
	5	5	2.301	0.117	2.312	-0.011
	6	5	1.819	0.108	1.824	-0.005

Note: The bolded value indicates the maximum deviation between the Observed Mean in log copies/mL and the Linear Fit Calculated Mean in log copies/mL.

The linearity study results for *C. dubliniensis* are presented in Table 16 to Table 18 below.

Table 16: Degree of Non-Linearity for *C. dubliniensis*

Best Fit Polynomial	Dilution #	N	Linear Fit Calculated Mean (Log copies/mL)	Best Fit Polynomial Calculated Mean (Log copies/mL)	Non-Linearity Best Fit Caculated Mean - Linear Fit Calculated Mean (Log copies/mL)	Degree of Non-Linearity is within ± 0.5 Log copies/mL (Yes or No)
2rd Degree Polynomial	1	5	5.040	4.886	-0.154	Yes
	2	5	4.470	4.501	0.031	Yes
	3	5	3.900	4.023	0.123	Yes
	4	5	3.330	3.453	0.123	Yes
	5	5	2.760	2.791	0.031	Yes
	6	5	2.190	2.035	-0.154	Yes

Table 17: Repeatability for *C. dubliniensis*

Candida Species	Dilution #	N	Observed Mean (Log copies/mL)	Observed Standatd Deviation (Log copies/mL)	Standard Deviation ≤ 0.25 Log copies/mL (Yes or No)
C. dubliniensis	1	5	4.937	0.018	Yes
	2	5	4.380	0.141	Yes
	3	5	4.117	0.088	Yes
	4	5	3.389	0.056	Yes
	5	5	2.868	0.138	Yes
	6	5	1.999	0.185	Yes

Table 18: *C. dubliniensis* –Deviations between the Observed Mean and the Linear Fit Polynomial Calculated Mean

Candida Species	Dilution #	N	Observed Mean (Log copies/mL)	Observed Standatd Deviation (Log copies/mL)	Linear Fit Calculated Mean (Log copies/mL)	Deviation (Observed Mean - Best Fit Polynomial Calculated Mean) (Log copies/mL)
C. dubliniensis	1	5	4.937	0.018	5.040	-0.103
	2	5	4.380	0.141	4.470	-0.090
	3	5	4.117	0.088	3.900	**0.217**
	4	5	3.389	0.056	3.330	0.059
	5	5	2.868	0.138	2.760	0.108
	6	5	1.999	0.185	2.190	-0.191

Note: The bolded value indicates the maximum deviation between the Observed Mean in log copies/mL and the Linear Fit Calculated Mean in log copies/mL.

C. tropicalis did not have a statistically significant 2nd or 3rd degree polynomial model fit, therefore, the linear fit was determined to be the best fit for this species. The linearity study results for *C. tropicalis* are presented in Table 19 and Table 20 below.

Table 19: Repeatability for *C. tropicalis*

Candida Species	Dilution #	N	Observed Mean (Log copies/mL)	Observed Standatd Deviation (Log copies/mL)	Standard Deviation ≤ 0.25 Log copies/mL (Yes or No)
C. tropicalis	1	5	4.839	0.066	Yes
	2	5	4.452	0.071	Yes
	3	5	3.801	0.167	Yes
	4	5	3.605	0.124	Yes
	5	5	2.987	0.036	Yes
	6	5	2.859	0.122	Yes

Table 20: *C. tropicalis* – Deviations between the Observed Mean and the Linear Fit Calculated Mean

***Candida* Species**	**Dilution #**	**N**	**Observed Mean (Log copies/mL)**	**Observed Standatd Deviation (Log copies/mL)**	**Linear Fit Calculated Mean (Log copies/mL)**	**Deviation Observed Mean - Best Fit Polynomial Calculated Mean (Log copies/mL)**
C. tropicalis	1	5	4.839	0.066	4.862	-0.023
	2	5	4.452	0.071	4.381	0.071
	3	5	3.801	0.167	3.936	-0.135
	4	5	3.605	0.124	3.455	0.150
	5	5	2.987	0.036	3.010	-0.023
	6	5	2.859	0.122	2.899	-0.040

Note: The bolded value indicates the maximum deviation between the Observed Mean in log copies/mL and the Linear Fit Calculated Mean in log copies/mL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

External Quality Controls

To generate valid results, a set of assay controls must be tested. One replicate each of negative control and positive control must be tested each time a reagent kit is loaded on the Panther system. Once established, the controls are valid for up to 24 hours. Software on the Panther system alerts the operator when controls are required. During processing, criteria for acceptance of controls are automatically verified by software on the Panther system. If any one of the controls has an invalid result, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

During the prospective clinical study conducted, a total of 55 positive controls and 55 negative controls were tested in valid runs on the Panther System. For the positive controls, a median TTime of 13.6 minutes with a range of 12.7 minutes to 16.4 minutes and a 4.85% CV were observed for the C spp (FAM) channel; a median TTime of 19.2 minutes with a range of 18.0 minutes to 28.6 minutes and a 9.84% CV were observed for the *C. glabrata* (HEX) channel; a median TTime of 17.3 minutes with a range of 16.3 minutes to 24.0 minutes and a 8.26% CV were observed for the *T. vaginalis* (ROX) channel; and a median TTime of 17.9 minutes with a range of 16.8 minutes to 26.6 minutes and a 10.31% CV were observed for the IC (Cy5.5) channel. For the negative controls, a median TTime of 17.0 minutes with a range of 16.0 minutes to 23.9 minutes and a 7.78% CV were observed for the IC (Cy5.5) channel.

Effectiveness of External Quality Control (Run Controls), Kit Calibrator and Internal Control

The Panther software enforces kit calibrator and run control addition after reagent kit loading and prior to processing samples. The assay also features an internal control. The kit calibrator, run controls, and the internal control are used to determine whether a run and/or a specimen result is valid. An analytical study was performed to assess whether the Aptima CV/TV assay run controls and/or calibration are properly

invalidated under operator-induced fault conditions that are not detected by instrument process controls.

The study results are presented in Table 21 below.

Table 21: List of Falt Conditions Tested, Run Validity Expectation, and Run Validity Result

Condition	Run Set Up	Run Validity Expection	Run Validity Result	Total Test [1]	Invalid [1] Tests	Run Errtor Codes
1	Normal Condition (Control)	Invalid	Invalid	80	80	NA
2	IC not added to TCR	Invalid	Invalid	80	80	p, x
3	Amplification reconstitution buffer used directly (no lyophilized reagent added)	Invalid	Invalid	80	80	f, p, x
4	Promotor reconstitution buffer used directly (no lyophilized reagent added)	Invalid	Invalid	80	80	f, p, x
5	Enzyme reconstitution buffer used directly (no lyophilized reagent added)	Invalid	Invalid	80	80	f, p, x, ebl
6	Amplification lyophilized reagent reconstituted with enzyme buffer; Enzyme lyophilized reagent reconstituted with Amplification buffer; Promoter reagent normal	Invalid	Invalid	80	80	f, p, x
7	Enzyme lyophilized reagent reconstituted with promoter buffer; Promoter lyophilized reagent reconstituted with enzyme buffer; Amplification reagent normal	Invalid	Invalid	80	80	f, p, x, FLSOR

f = calibration failed; p = assay processing error; x = control invalidated; ebl = the calculated estimated baseline is below the threshold value; FLSOR = a fluorometer has an off-target error

[1] Excluding kit calibrator and controls replicates.

The results of this study demonstrated that the Aptima CV/TV assay kit calibrators and controls are effective at properly invalidating operator induced fault conditions.

Vaginal Swab Specimen Stability

An analytical study was performed to establish the stability of clinical vaginal swab specimens for Aptima CV/TV assay testing. Clinical vaginal swab specimen stability was demonstrated by spiking whole organism lysate into individual naturally occurring negative vaginal swab specimens collected using the Aptima Multitest Swab Specimen Collection Kit, targeting 3x C_{95} or 3x LoD for each analyte. These specimens were tested with a minimum of 20 replicates under the following storage conditions:

- Storage at 2-8°C for a minimum of 30 days.
- Storage at 30°C for a minimum of 30 days.
- Storage at -20°C for a minimum of 60 days.
- Storage at 2-8°C for a minimum of 30 days followed by storage at -20°C for 60 days.
- Storage at 30°C for a minimum of 30 days followed by storage at -20°C for 60 days.

- Storage at -20°C through at least 3 freeze/thaw (F/T) cycles.

The vaginal swab specimen stability study results are presented in Table 22 below.

Table 22: Clinical Vaginal Swab Specimen Stability Results

Specimen	**Testing Time Point**	**Storage Conditions**	**Samples Tested (N)**	**% Aptima CV/TV Assay Positive**
C. albicans (3x C_{95})	T=0 (Baseline)	NA	24	100%
	T=30 Days	2-8°C	23	100%
		30°C	23	95.7% [1]
	T=60 Days	-20°C	23	100%
	T=90 Days	2-8°C for 30 days followed by -20°C for 60 days	23	100%
		30°C for 30 days followed by -20°C for 60 days	23	100%
	After three rounds of freeze/thaw	-20°C freeze/thaw	23	100%
C. glabrata (3x LoD)	T=0 (Baseline)	NA	24	100%
	T=30 Days	2-8°C	23	100%
		30°C	23	100%
	T=60 Days	-20°C	23	100%
	T=90 Days	2-8°C for 30 days followed by -20°C for 60 days	23	100%
		30°C for 30 days followed by -20°C for 60 days	23	100%
	After three rounds of freeze/thaw	-20°C freeze/thaw	23	100%
T. vaginalis (3x LoD)	T=0 (Baseline)	NA	24	100%
	T=30 Days	2-8°C	23	100%
		30°C	23	100%
	T=60 Days	-20°C	23	100%
	T=90 Days	2-8°C for 30 days followed by -20°C for 60 days	23	100%
		30°C for 30 days followed by -20°C for 60 days	23	100%
	After three rounds of freeze/thaw	-20°C freeze/thaw	23	100%

Note: At baseline, 24 samples were tested for each analyte. One *T. vaginalis* positive sample demonstrated background amplification for *C. albicans* in the FAM channel. All samples prepared using this natural negative donor were eliminated from further testing in this study, resulting in 23 samples being tested per subsequent time point per analyte.

[1] One sample was negative due to the log copy/mL value being below the C spp assay cutoff.

The clinical vaginal swab specimen stability study results met the acceptance criteria (i.e., ≥ 95% for all analytes at each condition evaluated) for this study for all conditions evaluated.

An additional analytical study was also performed to establish the stability of clinical vaginal swab specimens at 2-8°C for a minimum of 60 days for Aptima CV/TV assay testing. Clinical vaginal swab specimen stability at 2-8°C for a minimum of 60 days was demonstrated by spiking whole organism lysate into individual naturally occurring negative vaginal swab specimens collected using the Aptima Multitest Swab Specimen Collection Kit, targeting 3x C_{95} or 3x LoD for each analyte. These specimens were tested with 24 replicates.

Results of this additional vaginal swab specimen stability study also met the acceptance criteria; all replicates (100%) were detected for all analytes at the condition evaluated in this study.

Matrix Equivalency Study

A simulated vaginal swab matrix (SVSM) was developed for use in the prescision and reproducibility studies and several other analytical studies in order to mitigate the effect of Aptima CV/TV assay targeted analytes introduced by use of natural vaginal swab matrix (NVSM).

The SVSM consisted of 2.9 mL Aptima specimen transport medium (STM) combined with 150 uL of simulated vaginal fluid (SVF). The composition of the SVF is provided in Table 23 below.

Table 23: Simulated Vaginal Fluid (SVF) Composition

Composition (in water)	**Concentreation**
Sodium chloride	3.51 g/L
Potassium hydroxide	1.40 g/L
Calcium hydroxide	0.222 g/L
Bovine serum albumin	0.018 g/L
Lactic acid	2.00 g/L
Acetic acid	1.00 g/L
Glycerol	0.16 g/L
Urea	0.40 g/L
Glocose	5.00 g/L
C33A cells	33,000 cells/mL
pH 4.2	pH 4.2
Mucin	1.5% (w/v)

In order to utilize the SVSM as a simulated matrix in preparing contrived samples to be tested in precision/reproducibility studies and other analytical studies, an analytical study was performed to evaluate the performance using SVSM against a true negative NVSM with the Aptima CV/TV assay. Specimen Panels spiked with *C. albicans*, *C. glabrata*, or *T. vaginalis* lysate were prepared in both SVSM and NVSM at <1x, 1-2x, and 5x the LoD (for *C. glabrata* and *T. vaginalis*), or at <1x, 1-2x, and 5x the C_{95} (for *C. albicans*) concentrations. A minimum of 10 samples per spiking concentration per analyte for both SVSM and NVSM were tested with the Aptima CV/TV assay in this study.

Results of this study are presented in Table 24 below.

Table 24: Matrix Equivalency Study Results – SVSM vs. NVSM

Analyte	Concentration (multiples of LoD or C_{95})	Expected % Positive	N	Positive NVSM (N)	Positive SVSM (N)	% Positive NVSM	% Positive SVSM	Average TTime NVSM	Average TTime SVSM	Relevant Channel
C. albicans	Negative	0	10	0	0	0	0	NA	NA	FAM
	< 1x C_{95}	10-90	20	12	14	60%	70%	15.91	16.06	
	1-2x C_{95}	≥ 95	30	30	30	100%	100%	15.22	15.30	
	5x C_{95}	100	10	10	10	100%	100%	14.15	14.21	
C. glabrata	Negative	0	10	0	0	0	0	NA	NA	HEX
	< 1x C_{95}	10-90	10	3	4	30%	40%	22.87	22.78	
	1-2x C_{95}	≥ 95	30	30	30	100%	100%	22.08	21.65	
	5x C_{95}	100	10	10	10	100%	100%	19.95	19.80	
T. vaginalis	Negative	0	10	0	0	0	0	NA	NA	ROX
	< 1x C_{95}	10-90	10	1	6	10%	60%	27.70	27.68	
	1-2x C_{95}	≥ 95	30	30	30	100%	100%	25.00	23.79	
	5x C_{95}	100	10	10	10	100%	100%	22.60	22.06	

The data support equivalency between NVSM and SVSM for preparation of samples for the Aptima CV/TV assay analytical study and precision/reproducibility study.

Kit Calibration Interval

An analytical study was conducted to establish allowable kit calibration interval for the Aptima CV/TV assay on the Panther System.

The assay reagents were stored on-board the Panther instrument and tested at 0 (baseline), 24, and 30 hours using positive and negative samples. Results at 24 and 30 hours were analyzed using calibration established at the baseline.

The study results are presented in Table 25 below.

Table 25: Kit Calibration Study Results

Condition	Sample	Tested N	Reported Positive N	Reported Negative N	% Positive	Reported Valid N	Reported Invalid N	% Valid
0 Hour	Kit Calibrator	3	3	0	100%	3	0	100%
	Positive Control	1	1	0	100%	1	0	100%
	Negative Control	1	0	1	0%	1	0	100%
	Positive Control as Sample	5	5	0	100%	5	0	100%
	Negative Control as Sample	5	0	5	0%	5	0	100%
24 Hours	Positive Control as Sample	5	5	0	100%	5	0	100%
	Negative Control as Sample	5	0	5	0%	5	0	100%
30 Hours	Positive Control as Sample	5	5	0	100%	5	0	100%
	Negative Control as Sample	5	0	5	0%	5	0	100%

The study results showed no difference between testing at baseline (0 hour), 24 hours, and 30 hours after calibration, supporting the claim that Aptima CV/TV assay reagent kit calibration is valid for at least 24 hours when reagents are stored on-board the Panther System.

d. Detection limit:

The limit of detection (LoD) for each of the Aptima CV/TV assay analytes was determined by testing serial dilutions consisting of target organisms diluted in pooled negative natural vaginal swab matrix (NVSM) or simulated vaginal swab matrix (SVSM). A minimum of 20 replicates of each dilution were tested with each of the two reagent lots, for a minimum of 40 replicates per dilution. Probit analysis was performed to generate the 95% predicted detection limit for each organism. The predicted detection limits are shown in Table 26 below.

Table 26: Predicated Limit of Detection (LoD) of the Aptima CV/TV Assay by Probit Analysis

Organism	**Predicted Detection Limit**	**Concentration**	**Units**
Candida albicans [1]	95%	4439	CFU/mL
Candida glabrata [1]	95%	41	CFU/mL
Candida parapsilosis [2]	95%	9416	CFU/mL
Candida tropicalis [2]	95%	811	CFU/mL
Candida dubliniensis [2]	95%	1176	CFU/mL
Trichomonas vaginalis [1]	95%	0.0024	Cells/mL

[1] Tested in natural vaginal swab matrix (NVSM).
[2] Tested in simulated vaginal swab matrix (SVSM).

e. Analytical Reactivity

Five strains of each *Candida* target organism were tested using lysate targeting 3x LoD or C_{95} for *C. albicans, C. parapsilosis, C. tropicalis, C. dubliniensis,* and *C. glabrata* in SVSM. Nine strains of *T. vaginalis* including a metronidazole resistant strain were tested with cell lysate targeting 3x LoD in SVSM. The Aptima CV/TV assay was positive for all *Candida* strains tested at 3x LoD or C_{95}. Eight of the nine *T. vaginalis* strains, including the metronidazole resistant strain, were detected at 3x LoD. One strain of *T. vaginalis* was detected at 4x LoD.

Results of the analytical inclusivity study are presented in Table 27 below.

Table 27: Results of the Analytical Inclusivity Study

Species	**ATCC (Or Other Strain ID)**	**Replicates N**	**% Positive C spp (FAM)**	**% Positive *C. glabrata* (HEX)**	**% Positive *T. vaginalis* (ROX)**	**Test Concentration**
C. albicans	18804	20	100%	0%	0%	3x C_{95}
	14053	20	100%	0%	0%	3x C_{95}
	11006	20	100%	0%	0%	3x C_{95}
	24433	20	100%	0%	0%	3x C_{95}
	36232	20	100%	0%	0%	3x C_{95}
C. parapsilosis	22019	20	100%	0%	0%	3x C_{95}
	58895	20	100%	0%	0%	3x C_{95}
	7330	20	100%	0%	0%	3x C_{95}
	MYA-4646	20	100%	0%	0%	3x C_{95}
	96137	20	100%	0%	0%	3x C_{95}

C. tropicalis	750	20	100%	0%	0%	3x C_{95}
	42678	20	100%	0%	0%	3x C_{95}
	13803	20	100%	0%	0%	3x C_{95}
	34139	20	100%	0%	0%	3x C_{95}
	CDC: MAS92-384	20	100%	0%	0%	3x C_{95}
C. dubliniensis	MYA-580	20	100%	0%	0%	3x C_{95}
	MYA-582	20	100%	0%	0%	3x C_{95}
	MYA-581	20	100%	0%	0%	3x C_{95}
	MYA-583	20	100%	0%	0%	3x C_{95}
	MYA-180	20	100%	0%	0%	3x C_{95}
C. glabrata	2001	20	0%	100%	0%	3x LoD
	48435	20	0%	100%	0%	3x LoD
	CDC: MAS92-2	20	0%	100%	0%	3x LoD
	CDC: MAS92-115	20	0%	100%	0%	3x LoD
	CDC: MAS92-262	20	0%	100%	0%	3x LoD
T. vaginalis	30184	20	0%	0%	100%	3x LoD
	30185 [1]	20	0%	0%	100%	4x LoD
	30187	20	0%	0%	100%	3x LoD
	30188	20	0%	0%	100%	3x LoD
	30236	20	0%	0%	100%	3x LoD
	50143 [2]	20	0%	0%	100%	3x LoD
	30092	20	0%	0%	100%	3x LoD
	50144	20	0%	0%	100%	3x LoD
	50146	20	0%	0%	100%	3x LoD

[1] *T. vaginalis* ATCC 30185 passed the analytical reactivity criteria at 4x LoD.
[2] *T. vaginalis* strain ATCC 51043 is a metronidazole-resistant strain.

f. Analytical specificity

Cross-Reactivity Study

This study was performed to evaluate the potential of the Acucy Influenza A&B Test to cross-react with closely related and non-targeted microbial organisms, resulting in a false positive result. A total of 64 organisms and human cell lines prepared in SVSM were tested in triplicate with the Aptima CV/TV assay.

Results of the cross-reactivity study are summarized in Table 28 below.

Table 28: Summary Results of the Cross-Reactivity Study

Organism	**Final Concentration Tested**	**C spp Positive Replicates**	***C. glabrata* Positive Replicates**	***T. vaginalis* Positive Replicates**
Acinetobacter lwoffii	$1x10^6$ CFU/mL	0/3	0/3	0/3
Actinomyces israelii	$1x10^6$ CFU/mL	0/3	0/3	0/3
Alcaligenes faecalis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Atopobium vaginae	$1x10^6$ CFU/mL	0/3	0/3	0/3
Bacteroides fragilis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	0/3	0/3	0/3
BVAB-1 [1]	$1x10^6$ copies/mL	0/3	0/3	0/3
BVAB-2 [1]	$1x10^6$ copies/mL	0/3	0/3	0/3
Campylobacter jejuni	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida catenulata	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida famata [2]	$5x10^5$ CFU/mL	0/3	0/3	0/3
Candida guilliermondii	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida haemulonii	$1x10^6$ CFU/mL	0/3	0/3	0/3

Candida inconspicua	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida kefyr	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida krusei	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida lusitaniae	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida norvegica	$1x10^6$ CFU/mL	0/3	0/3	0/3
Candida orthopsilosis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Chlamydia trachomatis	$1x10^6$ IFU/mL	0/3	0/3	0/3
Clostridium difficile	$1x10^6$ CFU/mL	0/3	0/3	0/3
Corynebacterium genitalium	$1x10^6$ CFU/mL	0/3	0/3	0/3
Cryptococcus neoformans	$1x10^6$ CFU/mL	0/3	0/3	0/3
Eggerthella lenta	$1x10^6$ CFU/mL	0/3	0/3	0/3
Enterobacter cloacae	$1x10^6$ CFU/mL	0/3	0/3	0/3
Enterococcus faecalis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Escherichia coli	$1x10^6$ CFU/mL	0/3	0/3	0/3
Fusobacterium nucleatum	$1x10^6$ CFU/mL	0/3	0/3	0/3
Gardnerella vaginalis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Haemophilus ducreyi	$1x10^6$ CFU/mL	0/3	0/3	0/3
HeLa cells	$1x10^4$ Cells/mL	0/3	0/3	0/3
HIV	$1x10^5$ copies/mL	0/3	0/3	0/3
Herpes simplex virus 1	$1x10^4$ $TCID_{50}$/mL	0/3	0/3	0/3
Herpes simplex virus 2	$1x10^4$ $TCID_{50}$/mL	0/3	0/3	0/3
Klebsiella pneumoniae	$1x10^6$ CFU/mL	0/3	0/3	0/3
Lactobacillus acidophilus	$1x10^6$ CFU/mL	0/3	0/3	0/3
Lactobacillus crispatus	$1x10^6$ CFU/mL	0/3	0/3	0/3
Lactobacillus gasseri	$1x10^6$ CFU/mL	0/3	0/3	0/3
Lactobacillus iners	$1x10^6$ CFU/mL	0/3	0/3	0/3
Lactobacillus jensenii	$1x10^6$ CFU/mL	0/3	0/3	0/3
Lactobacillus mucosae	$1x10^6$ CFU/mL	0/3	0/3	0/3
Leptotrichia buccalis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Listeria monocytogenes	$1x10^6$ CFU/mL	0/3	0/3	0/3
Megasphaera Type 1 [1]	$1x10^6$ copies/mL	0/3	0/3	0/3
Mobiluncus curtisii	$1x10^6$ CFU/mL	0/3	0/3	0/3
Mycoplasma genitalium	$1x10^6$ CFU/mL	0/3	0/3	0/3
Mycoplasma hominis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Neisseria gonorrhoeae	$1x10^6$ CFU/mL	0/3	0/3	0/3
Peptostreptococcus magnus	$1x10^6$ CFU/mL	0/3	0/3	0/3
Pentatrichomonas hominis	$1x10^5$ cells/mL	0/3	0/3	0/3
Pichia fermentans	$1x10^6$ CFU/mL	0/3	0/3	0/3
Prevotella bivia	$1x10^6$ CFU/mL	0/3	0/3	0/3
Propionibacterium acnes	$1x10^6$ CFU/mL	0/3	0/3	0/3
Proteus vulgaris	$1x10^6$ CFU/mL	0/3	0/3	0/3
SiHa cells	$1x10^4$ cells/mL	0/3	0/3	0/3
Sneathia amnii	$1x10^6$ CFU/mL	0/3	0/3	0/3
Staphylococcus aureus	$1x10^6$ CFU/mL	0/3	0/3	0/3
Staphylococcus epidermidis	$1x10^6$ CFU/mL	0/3	0/3	0/3
Streptococcus agalactiae	$1x10^6$ CFU/mL	0/3	0/3	0/3
Streptococcus pyogenes	$1x10^6$ CFU/mL	0/3	0/3	0/3
Treponema pallidum [1]	$1x10^6$ copies/mL	0/3	0/3	0/3
Trichomonas tenax	$1x10^5$ cells/mL	0/3	0/3	0/3
Ureaplasma parvum	$1x10^6$ CFU/mL	0/3	0/3	0/3
Ureaplasma urealyticum	$1x10^6$ CFU/mL	0/3	0/3	0/3

CFU = Colony Forming Units; IFU = Inclusion Forming Units; $TCID_{50}$ = Median Tissue Culture Infectious Dose

[1] *In Vitro* Transcript tested.

[2] Cross-reactivity with *Candida famata* was seen at concentrations higher than $5x10^5$ CFU/mL.

No cross-reactivity was observed for any of the 64 organisms and human cell lines tested in the Aptima CV/TV assay at the concentrations listed in Table 28 above. Cross-reactivity with *Candida famata* was observed at concentrations higher than $5x10^5$ CFU/mL, and is noted in the Limitations section of the package insert.

Microbial Interference Study

Microbial interference was assessed for the Aptima CV/TV assay using the same 64-member test panel as the one used in the Cross-Reactivity Study above. A total of 64 organisms and human cell lines prepared in SVSM were tested in the presence of 3x LoD or C_{95} *C. albicans*, *C. glabrata* or *T. vaginalis* in triplicate with the Aptima CV/TV assay.

Results of the Microbial Interference Study are summarized in Table 29 below.

Table 29: Summary Results of the Microbial Interference Study

Organism	**Final Concentration Tested**	***C. albicans* (3xC_{95})**	***C. glabrata* (3xLoD)**	***T. vaginalis* (3xLoD)**
		C spp Positive Replicates	***C. glabrata* Positive Replicates**	***T. vaginalis* Positive Replicates**
Acinetobacter lwoffii	$1x10^6$ CFU/mL	3/3	3/3	3/3
Actinomyces israelii	$1x10^6$ CFU/mL	3/3	3/3	3/3
Alcaligenes faecalis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Atopobium vaginae	$1x10^6$ CFU/mL	3/3	3/3	3/3
Bacteroides fragilis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	3/3	3/3	3/3
BVAB-1 [1]	$1x10^6$ copies/mL	3/3	3/3	3/3
BVAB-2 [1]	$1x10^6$ copies/mL	3/3	3/3	3/3
Campylobacter jejuni	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida catenulata	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida famata	$5x10^5$ CFU/mL	3/3	3/3	3/3
Candida guilliermondii	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida haemulonii	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida inconspicua	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida kefyr	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida krusei	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida lusitaniae	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida norvegica	$1x10^6$ CFU/mL	3/3	3/3	3/3
Candida orthopsilosis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Chlamydia trachomatis	$1x10^6$ IFU/mL	3/3	3/3	3/3
Clostridium difficile	$1x10^6$ CFU/mL	3/3	3/3	3/3
Corynebacterium genitalium	$1x10^6$ CFU/mL	3/3	3/3	3/3
Cryptococcus neoformans	$1x10^6$ CFU/mL	3/3	3/3	3/3
Eggerthella lenta	$1x10^6$ CFU/mL	3/3	3/3	3/3
Enterobacter cloacae	$1x10^6$ CFU/mL	3/3	3/3	3/3
Enterococcus faecalis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Escherichia coli	$1x10^6$ CFU/mL	3/3	3/3	3/3
Fusobacterium nucleatum	$1x10^6$ CFU/mL	3/3	3/3	3/3
Gardnerella vaginalis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Haemophilus ducreyi	$1x10^6$ CFU/mL	3/3	3/3	3/3
HeLa cells	$1x10^4$ Cells/mL	3/3	3/3	3/3

HIV	$1x10^5$ copies/mL	3/3	3/3	3/3
Herpes simplex virus 1	$1x10^4$ $TCID_{50}$/mL	3/3	3/3	3/3
Herpes simplex virus 2	$1x10^4$ $TCID_{50}$/mL	3/3	3/3	3/3
Klebsiella pneumoniae	$1x10^6$ CFU/mL	3/3	3/3	3/3
Lactobacillus acidophilus	$1x10^6$ CFU/mL	3/3	3/3	3/3
Lactobacillus crispatus	$1x10^6$ CFU/mL	3/3	3/3	3/3
Lactobacillus gasseri	$1x10^6$ CFU/mL	3/3	3/3	3/3
Lactobacillus iners	$1x10^6$ CFU/mL	3/3	3/3	3/3
Lactobacillus jensenii	$1x10^6$ CFU/mL	3/3	3/3	3/3
Lactobacillus mucosae	$1x10^6$ CFU/mL	3/3	3/3	3/3
Leptotrichia buccalis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Listeria monocytogenes	$1x10^6$ CFU/mL	3/3	3/3	3/3
Megasphaera Type 1 [1]	$1x10^6$ copies/mL	3/3	3/3	3/3
Mobiluncus curtisii	$1x10^6$ CFU/mL	3/3	3/3	3/3
Mycoplasma genitalium	$1x10^6$ CFU/mL	3/3	3/3	3/3
Mycoplasma hominis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Neisseria gonorrhoeae	$1x10^6$ CFU/mL	3/3	3/3	3/3
Peptostreptococcus magnus	$1x10^6$ CFU/mL	3/3	3/3	3/3
Pentatrichomonas hominis	$1x10^5$ cells/mL	3/3	3/3	3/3
Pichia fermentans	$1x10^6$ CFU/mL	3/3	3/3	3/3
Prevotella bivia	$1x10^6$ CFU/mL	3/3	3/3	3/3
Propionibacterium acnes	$1x10^6$ CFU/mL	3/3	3/3	3/3
Proteus vulgaris	$1x10^6$ CFU/mL	3/3	3/3	3/3
SiHa cells	$1x10^4$ cells/mL	3/3	3/3	3/3
Sneathia amnii	$1x10^6$ CFU/mL	3/3	3/3	3/3
Staphylococcus aureus	$1x10^6$ CFU/mL	3/3	3/3	3/3
Staphylococcus epidermidis	$1x10^6$ CFU/mL	3/3	3/3	3/3
Streptococcus agalactiae	$1x10^6$ CFU/mL	3/3	3/3	3/3
Streptococcus pyogenes	$1x10^6$ CFU/mL	3/3	3/3	3/3
Treponema pallidum [1]	$1x10^6$ copies/mL	3/3	3/3	3/3
Trichomonas tenax	$1x10^5$ cells/mL	3/3	3/3	3/3
Ureaplasma parvum	$1x10^6$ CFU/mL	3/3	3/3	3/3
Ureaplasma urealyticum	$1x10^6$ CFU/mL	3/3	3/3	3/3

CFU = Colony Forming Units; IFU = Inclusion Forming Units; $TCID_{50}$ = Median Tissue Culture Infectious Dose

[1] *In Vitro* Transcript tested.

The results showed that the tested organisms and human cell lines at the concentrations tested in this study did not interfere with the detection of *C. albicans*, *C. glabrata*, or *T. vaginalis* samples at close to the LoD concentrations.

g. Competitive Interference Study

Since the Aptima CV/TV assay detects three analytes (C spp, *C. glabrata,* and *T. vaginalis*), a competitive interference study was conducted to determine whether target analytes at a high concentration would interfere with the detection of a second target analyte at near LoD concentrations in co-infected samples. Low concentration of one target lysate and high concentration of another target lysate in SVSM were tested in combination in 10 replicates per combination with the Aptima CV/TV assay.

Panel composition and concentrations are listed in Table 30 below.

Table 30: Co-Infection Test Panel

Panel Member	*C. albicans* Concentration	*C. glabrata* Concentration	*T. vaginalis* Concentration
C. albicans Low; *C. glabrata* High	3x C_{95}	$1x10^6$ CFU/mL	N/A
C. albicans Low; *T. vaginalis* High	3x C_{95}	N/A	$1x10^5$ cells/mL
C. glabrata Low; *T. vaginalis* High [1]	N/A	3x LoD	$1x10^3$ cells/mL
C. albicans High; *C. glabrata* Low	$1x10^6$ CFU/mL	3x LoD	N/A
C. albicans High; *T. vaginalis* Low	$1x10^6$ CFU/mL	N/A	3x LoD
C. glabrata High; *T. vaginalis* Low	N/A	$1x10^6$ CFU/mL	3x LoD

CFU = Colony Forming Units

[1] Competitive interference was observed for the combinations of low *C. glabrata* (3x LoD) and high *T. vaginalis* ($1x10^5$ cells/mL), and low *C. glabrata* (3x LoD) and high *T. vaginalis* ($1x10^4$ cells/mL). Further testing was conducted and resulted in 100% detection for the combination of low *C. glabrata* (3x LoD) and high *T. vaginalis* ($1x10^3$ cells/mL).

All testing resulted in 100% detection for both target analytes present except for the combination of low *C. glabrata* (3x LoD) and high *T. vaginalis* ($1x10^5$ or $1x10^4$ cells/mL). Further testing was conducted and resulted in 100% detection for the combination of low *C. glabrata* (3x LoD) and high *T. vaginalis* ($1x10^3$ cells/mL). The observed competitive interference is noted in the Limitations section of the package insert.

h. Potentially Interfering Substances Study

Potentially interfering substances were tested in the Aptima CV/TV assay. Panels were built in SVSM and evaluated in triplicate for potential effects on assay sensitivity and specificity. Sensitivity performance was evaluated separately for *C. albicans, C. glabrata*, and *T. vaginalis* by spiking lysate at 3x LoD (3x C_{95} for C spp). 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 31 below. Interference was observed in the presence of Tioconazole 6.5% Ointment, Vaginal Moisturizing Gel, and Glacial Acetic Acid, at higher concentrations (see Table 31 footnotes), and is noted in the Limitations section of the package insert.

Table 31: Interfering Substances Pabel

Substance	Final Concentration [1]
Whole Blood	5% V/V
Leukocytes	$1x10^6$ cells/mL
Mucus	5% V/V
Seminal Fluid	5% V/V
Contraceptive Foam	5% W/V
Contraceptive Film	5% W/V
Tioconazole 6.5% [2]	2% 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 [3]	0.5% W/V
Lubricant	5% V/V
Spermicide	5% W/V
Anti-fungal	5% W/V
Deodorant/Spray	5% W/V
Glacial Acetic Acid [4]	4% 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] Tioconazole 6.5% Ointment: Interference was observed at ≥ 3% W/V for all analytes. No interference was observed at 2% W/V for all analytes.

[3] Vaginal Moisturizing Gel: Interference was observed at 5% W/V for *C. glabrata*, ≥ 3% W/V for *T. vaginalis*, and ≥ 1% W/V for *C. albicans*. No interference was observed at 0.5% W/V for *C. albicans*, 4% W/V for *C. glabrata*, and 2% W/V for *T. vaginalis*.

[4] Glacial Acetic Acid: Interference was observed at 5% V/V for *C. albicans*. No interference was observed at 4% V/V for *C. albicans*, 5% V/V for *C. glabrata*, and 5% V/V for *T. vaginalis*.

i. Carry-Over Study

Since carry-over rates on the Panther System had already been established for several other FDA cleared assays, such as the Aptima *Trichomonas vaginalis* Assay (K122062) and the Aptima Combo 2 Assay (K132251 and K111409), no additional testing on instrument carryover/cross-contamination for the Aptima CV/TV assay on the fully automated Panther System was performed.

j. Assay cut-off

Minimum Specifications for an Amplified Reaction

For Aptima CV/TV assay amplification, the *C. glabrata* (HEX) and *T. vaginalis* (ROX) minimum specifications for an amplified reaction also serve as the specifications for a positive reaction. For C spp (FAM), these channel minimum specifications for an amplified reaction serve as the specifications for a positive reaction only when the sample is co-infected with a high concentration of *T. vaginalis* exceeding a predetermined threshold (as determined by TTime in the ROX channel).

The parameters that determine a legitimate amplified reaction for each channel are as follows:

- RFU Range – Relative Fluorescent Unit (RFU) is a measure of the intensity of the fluorescent signal, specific for each channel. The RFU Range is the difference between maximum and minimum fluorescent signal seen in a sample during amplification. The RFU range specification for each channel represents the minimum fluorescent threshold for an amplification curve to generate a TTime.
- TTime Max – The TTime parameter is a measure of the time (in minutes) for the real-time amplified fluorescent curve to emerge from the background signal. TTime values are generated based on the normalized curve from the Panther System TTime algorithm. The TTime max value is the maximum amount of time allowed for an amplification curve to generate a TTime. If the TTime exceeds this threshold, amplification has not occurred, and the specimen is negative.
- Emergence Rate - Emergence Rate is the background subtracted slope of the emerging fluorescent curve, calculated by linear regression of N data points before and after the established TTime.

The minimum specifications for an amplified reaction for each of the four channels are presented in Table 32 below.

Table 32: Specifications for an Amplified Reaction

Analyte	Channel	RFU Range	TTime Max	Emergence Rate
C spp	FAM	1000	45	40
C. glabrata	HEX	1000	45	50
T. vaginalis	ROX	1000	50	80
Internal Control	Cy5.5	1000	50	30

To determine the appropriate minimum specifications for an amplified reaction, logistic regression and receiver operating characteristic (ROC) analyses were initially performed with data generated from a minimum of 635 positive and a minimum of 594 negative samples for each channel during assay development. All parameter values were subsequently validated during assay development by running an independent set of 617 clinical samples with the proposed specifications and analyzed relative to composite culture and bi-directional sequencing comparator or NAAT assay comparator results to ensure the selected values maximized assay sensitivity and specificity. The selected specifications were further validated during the prospective clinical study and the contrived clinical specimens testing.

Assay Cut-off for the C spp (FAM) Channel

During Aptima CV/TV assay development, preliminary analysis of the C spp assay results compared to *Candida* culture showed that the Limit of Detection (LoD) for the

C spp assay (FAM) was sensitive enough to detect low level *Candida* infections; i.e. negative by culture (culture status below +1). The reference used for *Candida* species group positivity is *Candida* culture with Sabouraud Dextrose Agar and CHROMagar with +1 culture or above (on a scale of +1 to +4). An assay cutoff for the C spp assay (FAM) was therefore developed to better align the Aptima CV/TV assay detection of C spp with the comparator method of culture in order to achieve the desired specificity.

A calibration system was developed for the C spp assay (FAM channel) in order to optimize the C spp assay cutoff and adjust for run-to-run differences. Prior to establishing the C spp assay cutoff during early assay development, the specificity of the C spp assay against *Candida* culture was less than 90% in 430 clinical samples. The C spp assay cutoff was developed by running clinical sample collections with known culture status along with standards of known concentration (*in vitro* transcript calibrators). Linear regression was performed with the calibration standards and the TTimes of each characterized sample converted to a relative unit of log copies/mL. These relative units were compared against culture results to select the assay cutoff (in log copies/mL) which improved the C spp assay specificity while still providing acceptable assay sensitivity.

To verify the selected cutoff was appropriate relative to the *Candida* culture comparator, an independent set of 617 clinical samples with known *Candida* culture status was tested. The C spp assay (FAM channel) achieved a 92.8% sensitivity and 94.2% specificity in this cohort of clinical samples with the implemented C spp assay cutoff of > 2.38 log copies/mL. The selected C spp assay cut-off was further validated during the prospective clinical study.

2. Comparison studies:

   a. Method comparison with predicate device:

   Not applicable.

   b. Matrix comparaison:

   Not applicable.

3. Clinical Studies:

   Prospective Clinical Study

   A prospective, multi-center clinical study was conducted to establish the clinical performance characteristics of the Aptima CV/TV assay on the Panther system. Female subjects, ≥14 years of age, presenting with symptoms of vaginitis, were enrolled at 21 geographically and ethnically diverse US clinical sites, including private and academic

family practice, obstetric-gynecologic, family planning, public health, sexually transmitted infections (STI), and medical group clinics, and clinical research centers.

Five (5) vaginal swab samples were collected from each subject during the prospective clinical study: one clinician-collected swab sample and one patient-collected swab sample were collected using the Aptima Multitest Swab Specimen Collection Kit for Aptima CV/TV assay testing, and three additional vaginal swab samples were collected for reference testing, including one patient-collected swab sample for testing using an FDA-cleared molecular assay for *T. vaginalis*, one clinician-collected swab sample for testing using an FDA-cleared culture-based assay for *T. vaginalis*, and one clinician-collected swab sample for *Candida* culture testing.

Two (2) collected vaginal swab samples, one patient-collected and one clinician-collected, using the Aptima Multitest Swab Specimen Collection Kit (referred to as the Aptima swab samples hereafter) were shipped to an accessioning center prior to being distributed and tested with the Aptima CV/TV assay on the Panther System at three testing sites.

Candida species group (C spp) and *C. glabrata* infection status were determined seperately using a combination of *Candida* culture (using both the Sabouraud dextrose agar method and the chromogenic agar method), followed by PCR/bi-directional sequencing of both Aptima swab samples leftover after testing with the Aptima CV/TV assay, if *Candida* growth was observed on either culture plate. Growth of any *Candida* on either culture plate was interpreted as a positive culture result, and absence of growth of *Candida* on both culture plates was interpreted as a negative culture result, hence a reference negative result for both C spp and *C. glabrata*. For subjects with positive culture growth (i.e., *Candida* growth on either culture plate), both Aptima swab samples leftover after testing with the Aptima CV/TV assay were used for PCR/bi-directional sequencing to determine whether C spp or *C. glabrata* were present. C spp identification by PCR/bi-directional sequencing in any one or both of the Aptima swab samples was used as the positive reference result for C spp for both Aptima swab types (patient-collected and one clinician-collected), and no C spp detected by PCR/bi-directional sequencing in both of the Aptima swab samples was used as the negative reference result for C spp for both Aptima swab types. Similarly, *C. glabrata* identification by PCR/bi-directional sequencing in any one or both of the Aptima swab samples was used as the positive reference result for *C. glabrata* for both Aptima swab types, and no *C. glabrata* detected by PCR/bi-directional sequencing in both of the Aptima swab samples was used as the negative reference result for *C. glabrata* for both Aptima swab types.

T. vaginalis patient infection status (PIS) was determined using a composite result from two FDA-cleared assays for *T. vaginalis*, one molecular assay and one culture-based assay. *T. vaginalis* detection by any one or both of the FDA-cleared assays was used as the positive reference result for *T. vaginalis* for both Aptima swab types (patient-collected and one clinician-collected), and no *T. vaginalis* detected by both FDA-cleared assays was used as the negative reference result for *T. vaginalis* for both Aptima swab types.

Aptima CV/TV assay performance characteristics for each prospectively-collected specimen type, with corresponding 2-sided 95% Score confidence intervals (CIs), were estimated relative to the C spp and *C. glabrata* reference results and *T. vaginalis* PIS as described previously.

Of the 1519 symptomatic subjects enrolled in the clinical study, 17 subjects were withdrawn, and six subjects were not evaluable due to final invalid Aptima CV/TV assay results (n = 1), missing vaginal swabs (n = 1), or unknown C spp infection status or *T. vaginalis* PIS (n = 4). The remaining 1496 subjects were evaluable for at least one analyte in at least one of the specimen types. Table 33 below shows the demographics of evaluable subjects.

Table 33: Demographics of Evaluable Symptomatic Subjects in the Prospective Clinical Evaluation of the Aptima CV/TV Assay

Total Evaluable Subjects	1496
Age (years)	
Mean ± SD	35.3 ± 11.76
Median	33.0
Range	14 - 79
Age category (years)	
14-17	5 (0.3%)
18-29	554 (37.0%)
30-39	480 (32.1%)
40-49	247 (16.5%)
>50	210 (14.0%)
Race/Ethnicity	
Asian	73 (4.9%)
Black or African American	752 (50.3%)
White (Hispanic or Latino)	268 (17.9%)
White (Not Hispanic or Latino)	339 (22.7%)
Other [1]	64 (4.3%)

[1] Includes patient-reported “other”, “mixed”, and unknown races.

For the 1496 evaluable subjects, 1485 clinician-collected vaginal swab samples and 1477 patient-collected vaginal swab samples were included in the analyses for C spp; 1483 clinician-collected vaginal swab samples and 1475 patient-collected vaginal swab samples were included in the analyses for *C. glabrata*; and 1438 clinician-collected vaginal swab samples and 1433 patient-collected vaginal swab samples were included in the analyses for *T. vaginalis*.

C spp Performance

Table 34 below includes overall and per collection site performance for reporting C spp as observed in the prospective clinical study. The sensitivity and specificity for C spp

were 91.7% and 94.9%, respectively, for clinician-collected vaginal swabs, and 92.9% and 91.0%, respectively, for patient-collected vaginal swabs. For the population tested, this resulted in Positive Predictive Values (PPV) of 87.8% and 80.5% for clinician-collected and patient-collected specimens, respectively. Negative Predictive Values (NPV) of 96.6 % and 97.0% were obtained for clinician-collected and patient-collected specimens, respectively.

Table 34: C spp Performance by Specimen Collection Type and Specimen Collection Site

Site	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
Overall	**1485**	**28.6**	**91.7 (88.7-94.0) 389/424**	**94.9 (93.4-96.1) 1007/1061**	**1477**	**28.6**	**92.9 (90.0-95.0) 392/422**	**91.0 (89.1-92.6) 960/1055**
#1	20	25.0	60.0 (23.1-88.2) 3/5	100 (79.6-100) 15/15	20	25.0	60.0 (23.1-88.2) 3/5	93.3 (70.2-98.8) 14/15
#2	5	0.0	NC	80.0 (37.6-96.4) 4/5	5	0.0	NC	100 (56.6-100) 5/5
#3	22	54.5	91.7 (64.6-98.5) 11/12	90.0 (59.6-98.2) 9/10	22	54.5	91.7 (64.6-98.5) 11/12	90.0 (59.6-98.2) 9/10
#4	216	22.2	85.4 (72.8-92.8) 41/48	94.6 (90.1-97.2) 159/168	213	22.5	85.4 (72.8-92.8) 41/48	88.5 (82.7-92.5) 146/165
#5	147	24.5	88.9 (74.7-95.6) 32/36	94.6 (88.7-97.5) 105/111	144	24.3	91.4 (77.6-97.0) 32/35	91.7 (85.0-95.6) 100/109
#6	72	31.9	100 (85.7-100) 23/23	98.0 (89.3-99.6) 48/49	72	31.9	95.7 (79.0-99.2) 22/23	95.9 (86.3-98.9) 47/49
#7	197	21.8	93.0 (81.4-97.6) 40/43	94.8 (90.1-97.3) 146/154	197	21.8	90.7 (78.4-96.3) 39/43	89.6 (83.8-93.5) 138/154
#8	1	0.0	NC	100 (20.1-100) 1/1	1	0.0	NC	100 (20.7-100) 1/1
#9	108	43.5	87.2 (74.8-94.0) 41/47	100 (94.1-100) 61/61	108	43.5	93.6 (82.8-97.8) 44/47	90.2 (80.2-95.4) 55/61
#10	17	35.3	100 (61.0-100) 6/6	81.8 (52.3-94.9) 9/11	17	35.3	100 (61.0-100) 6/6	72.7 (43.4-90.3) 8/11
#11	71	26.8	89.5 (68.6-97.1) 17/19	96.2 (87.0-98.9) 50/52	72	26.4	94.7 (75.4-99.1) 18/19	96.2 (87.2-99.0) 51/53
#12	138	31.9	95.5 (84.9-98.7) 42/44	95.7 (89.6-98.3) 90/94	135	31.1	95.2 (84.2-98.7) 40/42	93.5 (86.6-97.0) 87/93
#13	69	27.5	100 (83.2-100) 19/19	96.0 (86.5-98.9) 48/50	69	29.0	95.0 (76.4-99.1) 19/20	93.9 (83.5-97.9) 46/49

#14	9	44.4	100 (51.0-100) 4/4	100 (56.6-100) 5/5	9	44.4	100 (51.0-100) 4/4	100 (56.6-100) 5/5
#15	4	50.0	100 (34.2-100) 2/2	100 (34.2-100) 2/2	4	50.0	100 (34.2-100) 2/2	100 (34.2-100) 2/2
#16	30	43.3	84.6 (57.8-95.7) 11/13	94.1 (73.0-99.0) 16/17	30	43.3	92.3 (66.7-98.6) 12/13	88.2 (65.7-96.7) 15/17
#17	80	35.0	92.9 (77.4-98.0) 26/28	92.3 (81.8-97.0) 48/52	80	35.0	96.4 (82.3-99.4) 27/28	90.4 (79.4-95.8) 47/52
#18	86	30.2	92.3 (75.9-97.9) 24/26	88.3 (77.8-94.2) 53/60	86	30.2	96.2 (81.1-99.3) 25/26	88.3 (77.8-94.2) 53/60
#19	75	41.3	100 (89.0-100) 31/31	95.5 (84.9-98.7) 42/44	75	41.3	100 (89.0-100) 31/31	88.6 (76.0-95.0) 39/44
#20	39	7.7	100 (43.9-100) 3/3	97.2 (85.8-99.5) 35/36	39	7.7	100 (43.9-100) 3/3	97.2 (85.8-99.5) 35/36
#21	79	19.0	86.7 (62.1-96.3) 13/15	95.3 (87.1-98.4) 61/64	79	19.0	86.7 (62.1-96.3) 13/15	89.1 (79.1-94.6) 57/64

NC = Not Calculated
[1] Score 95% Confidence Interval.

Tables 35, 36 and 37 below include C spp performance for clinician-collected and patient-collected vaginal specimens stratified respectively by age group, ethnicity, and patient clinical condition.

Table 35: C spp Performance by Age Group

Age Group	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
All	**1485**	**28.6**	**91.7 (88.7-94.0) 389/424**	**94.9 (93.4-96.1) 1007/1061**	**1477**	**28.6**	**92.9 (90.0-95.0) 392/422**	**91.0 (89.1-92.6) 960/1055**
14-17	5	40.0	100.0 (34.2-100.0) 2/2	100.0 (43.9-100.0) 3/3	5	40.0	100.0 (34.2-100.0) 2/2	100.0 (43.9-100.0) 3/3
18-29	549	31.9	93.7 (89.1-96.5) 164/175	94.9 (92.2-96.7) 355/374	548	31.9	94.3 (89.8-96.9) 165/175	91.4 (88.1-93.9) 341/373
30-39	475	29.9	91.5 (85.8-95.1) 130/142	94.0 (90.9-96.1) 313/333	472	29.9	93.6 (88.3-96.6) 132/141	91.5 (88.0-94.1) 303/331
40-49	246	26.0	90.6 (81.0-95.6) 58/64	95.6 (91.6-97.8) 174/182	245	26.1	90.6 (81.0-95.6) 58/64	90.6 (85.5-94.1) 164/181
≥50	210	19.5	85.4 (71.6-93.1) 35/41	95.9 (91.7-98.0) 162/169	207	19.3	87.5 (73.9-94.5) 35/40	89.2 (83.6-93.1) 149/167

NC = Not Calculated
[1] Score 95% Confidence Interval.

Table 36: C spp Performance by Ethnicity

Ethnicity	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
All	**1485**	**28.6**	**91.7 (88.7-94.0) 389/424**	**94.9 (93.4-96.1) 1007/1061**	**1477**	**28.6**	**92.9 (90.0-95.0) 392/422**	**91.0 (89.1-92.6) 960/1055**
Asian	73	26.0	100 (83.2-100) 19/19	94.4 (84.9-98.1) 51/54	71	25.4	100 (82.4-100) 18/18	90.6 (79.7-95.9) 48/53
Black/African-American	747	30.4	90.7 (86.3-93.9) 206/227	94.0 (91.7-95.8) 489/520	745	30.6	90.8 (86.3-93.9) 207/228	89.4 (86.4-91.7) 462/517
White (Hispanic/Latino)	265	28.7	93.4 (85.5-97.2) 71/76	93.7 (89.2-96.3) 177/189	265	28.7	93.4 (85.5-97.2) 71/76	89.9 (84.8-93.5) 170/189
White (Non Hispanic/Latino)	336	23.8	91.3 (83.0-95.7) 73/80	97.7 (95.0-98.9) 250/256	332	23.5	96.2 (89.3-98.7) 75/78	95.3 (91.9-97.3) 242/254
Other [2]	64	34.4	90.9 (72.2-97.5) 20/22	95.2 (84.2-98.7) 40/42	64	34.4	95.5 (78.2-99.2) 21/22	90.5 (77.9-96.2) 38/42

NC = Not Calculated

[1] Score 95% Confidence Interval.

[2] Includes patient-reported “other”, “mixed”, and unknown races

Table 37: C spp Performance by Clinical Condition

Clinical Condition	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
Use of antibiotics	5	60.0	66.7 (20.8-93.9) 2/3	50.0 (9.5-90.5) 1/2	5	60.0	66.7 (20.8-93.9) 2/3	0.0 (0.0-65.8) 0/2
Use of antifungals	8	37.5	100 (43.9-100) 3/3	100 (56.6-100) 5/5	8	37.5	100 (43.9-100) 3/3	100 (56.6-100) 5/5
Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2	2	0.0	NC	100 (34.2-100) 2/2
Recurrent symptoms of vaginitis in the last 12 months	863	28.6	89.9 (85.5-93.0) 222/247	95.0 (92.9-96.4) 585/616	859	28.6	90.7 (86.4-93.7) 223/246	91.2 (88.7-93.2) 559/613
Unprotected intercourse in the last 24 hours	96	27.1	84.6 (66.5-93.8) 22/26	92.9 (84.3-96.9) 65/70	95	27.4	88.5 (71.0-96.0) 23/26	85.5 (75.3-91.9) 59/69
Pregnant	20	55.0	100 (74.1-100) 11/11	100 (70.1-100) 9/9	21	52.4	100 (74.1-100) 11/11	100 (72.2-100) 10/10
With menses	118	30.5	94.4 (81.9-98.5) 34/36	97.6 (91.5-99.3) 80/82	116	30.2	97.1 (85.5-99.5) 34/35	88.9 (80.2-94.0) 72/81
Without menses	1210	29.6	92.5 (89.2-94.8) 331/358	94.4 (92.6-95.7) 804/852	1207	29.7	93.0 (89.9-95.2) 333/358	91.0 (88.9-92.8) 773/849
Post-menopausal	157	19.1	80.0 (62.7-90.5) 24/30	96.9 (92.2-98.8) 123/127	154	18.8	86.2 (69.4-94.5) 25/29	92.0 (85.9-95.6) 115/125

NC = Not Calculated

[1] Score 95% Confidence Interval.

C. glabrata Performance

Table 38 below includes overall and per collection site performance for reporting *C. glabrata* as observed in the prospective clinical study. The sensitivity and specificity for *C. glabrata* were 84.7% and 99.1%, respectively, for clinician-collected vaginal swabs, and 86.2% and 98.7%, respectively, for patient-collected vaginal swabs. For the population tested, this resulted in Positive Predictive Values (PPV) of 79.4% and 73.5% for clinician-collected and patient-collected specimens, respectively. Negative Predictive Values (NPV) of 99.4 % was obtained for both clinician-collected and patient-collected specimens.

Table 38: *C. glabrata* Performance by Specimen Collection Type and Specimen Collection Site

Site	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
Overall	**1483**	**4.0**	**84.7 (73.5-91.8) 50/59 [2]**	**99.1 (98.4-99.5) 1411/1424 [3]**	**1475**	**3.9**	**86.2 (75.1-92.8) 50/58 [4]**	**98.7 (98.0-99.2) 1399/1417 [5]**
#1	20	5.0	100 (20.7-100) 1/1	100 (83.2-100) 19/19	20	5.0	100 (20.7-100) 1/1	100 (83.2-100) 19/19
#2	5	0.0	NC	100 (56.6-100) 5/5	5	0.0	NC	100 (56.6-100) 5/5
#3	22	0.0	NC	100 (85.1-100) 22/22	22	0.0	NC	100 (85.1-100) 22/22
#4	215	5.6	66.7 (39.1-86.2) 8/12	98.5 (95.7-99.5) 200/203	213	5.6	75.0 (46.8-91.1) 9/12	97.0 (93.6-98.6) 195/201
#5	147	4.8	100 (64.6-100) 7/7	100 (97.3-100) 140/140	144	4.9	100 (64.6-100) 7/7	99.3 (96.0-99.9) 136/137
#6	72	2.8	100 (34.2-100) 2/2	98.6 (92.3-99.7) 69/70	72	2.8	100 (34.2-100) 2/2	98.6 (92.3-99.7) 69/70
#7	197	7.1	71.4 (45.4-88.3) 10/14	97.3 (93.8-98.8) 178/183	197	7.1	71.4 (45.4-88.3) 10/14	97.8 (94.5-99.1) 179/183
#8	1	0.0	NC	100 (20.1-100) 1/1	1	0.0	NC	100 (20.7-100) 1/1
#9	108	1.9	100 (34.2-100) 2/2	100 (96.5-100) 106/106	108	1.9	100 (34.2-100) 2/2	99.1 (94.8-99.8) 105/106
#10	17	5.9	100 (20.7-100) 1/1	100 (80.6-100) 16/16	17	5.9	100 (20.7-100) 1/1	100 (80.6-100) 16/16
#11	71	4.2	100 (43.9-100) 3/3	98.5 (92.1-99.7) 67.68	72	4.2	100 (43.9-100) 3/3	98.6 (92.2-99.7) 68/69
#12	138	2.9	100 (51.0-100) 4/4	100 (97.2-100) 134/134	135	2.2	100 (43.9-100) 3/3	99.2 (95.8-99.9) 131/132

#13	69	1.4	100 (20.7-100) 1/1	100 (94.7-100) 68/68	68	1.5	100 (20.7-100) 1/1	98.5 (92.0-99.7) 66/67
#14	9	0.0	NC	100 (70.1-100) 9/9	9	0.0	NC	100 (70.1-100) 9/9
#15	4	0.0	NC	100 (51.0-100) 4/4	4	0.0	NC	100 (51.0-100) 4/4
#16	30	0.0	NC	96.7 (83.3-99.4) 29/30	30	0.0	NC	96.7 (83.3-99.4) 29/30
#17	80	2.5	50.0 (9.5-90.5) 1/2	98.7 (93.1-99.8) 77/78	80	2.5	50.0 (9.5-90.5) 1/2	100 (95.3-100) 78/78
#18	85	1.2	100 (20.7-100) 1/1	100 (95.6-100) 84/84	85	1.2	100 (20.7-100) 1/1	100 (95.6-100) 84/84
#19	75	5.3	100 (51.0-100) 4/4	100 (94.9-100) 71/71	75	5.3	100 (51.0-100) 4/4	100 (94.9-100) 71/71
#20	39	5.1	100 (34.2-100) 2/2	100 (90.6-100) 37/37	39	5.1	100 (34.2-100) 2/2	100 (90.6-100) 37/37
#21	79	3.8	100 (43.9-100) 3/3	98.7 (92.9-99.8) 75/76	79	3.8	100 (43.9-100) 3/3	98.7 (92.9-99.8) 75/76

NC = Not Calculated

[1] Score 95% Confidence Interval.

[2] All 9 samples with false negative results showed no growth of *C. glabrata* on chromogenic agar.

[3] Of the 13 samples with false positive results, 2 showed high (4+) growth, 2 showed low (≤2+) growth, and 9 showed no growth of *C. glabrata* on chromogenic agar.

[4] Of the 8 samples with false negative results, 7 showed no growth and 1 showed high (4+) growth of *C. glabrata* on chromogenic agar.

[5] Of the 18 samples with false positive results, 2 showed high (4+) growth, 2 showed low (≤2+) growth, and 14 showed no growth of *C. glabrata* on chromogenic agar.

Tables 39, 40 and 41 below include *C. glabrata* performance for clinician-collected and patient-collected vaginal specimens stratified respectively by age group, ethnicity, and patient clinical condition.

Table 39: *C. glabrata* Performance by Age Group

Age Group	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
All	**1483**	**4.0**	**84.7 (73.5-91.8) 50/59**	**99.1 (98.4-99.5) 1411/1424**	**1475**	**3.9**	**86.2 (75.1-92.8) 50/58**	**98.7 (98.0-99.2) 1399/1417**
14-17	5	0.0	NC	100.0 (56.6-100.0) 5/5	5	0.0	NC	100.0 (56.6-100.0) 5/5
18-29	549	2.4	76.9 (49.7-91.8) 10/13	99.4 (98.4-99.8) 533/536	547	2.4	76.9 (49.7-91.8) 10/13	99.3 (98.1-99.7) 530/534
30-39	473	2.7	84.6 (57.8-95.7) 11/13	99.6 (98.4-99.9) 458/460	471	2.8	84.6 (57.8-95.7) 11/13	99.1 (97.8-99.7) 454/458
40-49	246	7.3	83.3 (60.8-94.2) 15/18	99.1 (96.9-99.8) 226/228	245	7.3	88.9 (67.2-96.9) 16/18	99.1 (96.8-99.8) 225/227
≥50	210	7.1	93.3 (70.2-98.8) 14/15	96.9 (93.5-98.6) 189/195	207	6.8	92.9 (68.5-98.7) 13/14	95.9 (92.0-97.9) 185/193

NC = Not Calculated

[1] Score 95% Confidence Interval.

Table 40: *C. glabrata* Performance by Ethnicity

Ethnicity	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
All	**1483**	**4.0**	**84.7 (73.5-91.8) 50/59**	**99.1 (98.4-99.5) 1411/1424**	**1475**	**3.9**	**86.2 (75.1-92.8) 50/58**	**98.7 (98.0-99.2) 1399/1417**
Asian	72	4.2	100 (43.9-100) 3/3	100 (94.7-100) 69/69	71	4.2	100 (43.9-100) 3/3	98.5 (92.1-99.7) 67/68
Black/African-American	747	4.1	74.2 (56.8-86.3) 23/31	98.7 (97.6-99.3) 707/716	745	4.2	77.4 (60.2-88.6) 24/31	98.7 (97.6-99.3) 704/713
White (Hispanic/Latino)	264	3.0	87.5 (52.9-97.8) 7/8	99.6 (97.8-99.9) 255/256	265	3.0	87.5 (52.9-97.8) 7/8	99.2 (97.2-99.8) 254/256
White (Non Hispanic/Latino)	336	4.2	100 (78.5-100) 14/14	99.1 (97.3-99.7) 319/322	332	3.9	100 (77.2-100) 13/13	98.4 (96.4-99.3) 314/319
Other [2]	64	4.7	100 (43.9-100) 3/3	100 (94.1-100) 61/61	64	4.7	100 (43.9-100) 3/3	98.4 (91.3-99.7) 60/61

NC = Not Calculated

[1] Score 95% Confidence Interval.

[2] Includes patient-reported “other”, “mixed”, and unknown races

Table 41: *C. glabrata* Performance by Clinical Condition

Clinical Condition	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
Use of antibiotics	5	20.0	100 (20.7-100) 1/1	100 (51.0-100) 4/4	5	20.0	100 (20.7-100) 1/1	100 (51.0-100) 4/4
Use of antifungals	8	12.5	100 (20.7-100) 1/1	100 (64.6-100) 7/7	8	12.5	100 (20.7-100) 1/1	100 (64.6-100) 7/7
Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2	2	0.0	NC	100 (34.2-100) 2/2
Recurrent symptoms of vaginitis in the last 12 months	861	3.9	88.2 (73.4-95.3) 30/34	99.0 (98.1-99.5) 819/827	859	4.0	91.2 (77.0-97.0) 31/34	99.2 (98.3-99.6) 817/824
Unprotected intercourse in the last 24 hours	96	4.2	100 (51.0-100) 4/4	100 (96.0-100) 92/92	95	4.2	100 (51.0-100) 4/4	100 (95.9-100) 91/91
Pregant	20	0.0	NC	95.0 (76.4-99.1) 19/20	21	0.0	NC	90.5 (71.1-97.3) 19/21
With menses	117	2.6	100 (43.9-100) 3/3	100 (96.7-100) 114/114	116	2.6	100 (43.9-100) 3/3	100 (96.7-100) 113/113
Without menses	1209	3.8	80.4 (66.8-89.3) 37/46	99.1 (98.4-99.5) 1153/1163	1205	3.8	84.8 (71.8-92.4) 39/46	99.0 (98.2-99.4) 1147/1159
Post-menopausal	157	6.4	100 (72.2-100) 10/10	98.0 (94.2-99.3) 144/147	154	5.8	88.9 (56.5-98.0) 8/9	95.9 (91.3-98.1) 139/145

NC = Not Calculated
[1] Score 95% Confidence Interval.

T. vaginalis Performance

Table 42 below includes overall and per collection site performance for reporting *T. vaginalis* as observed in the prospective clinical study. The sensitivity and specificity for *T. vaginalis* were 96.5% and 95.1%, respectively, for clinician-collected vaginal swabs, and 97.1% and 98.9%, respectively, for patient-collected vaginal swabs. For the population tested, this resulted in Positive Predictive Values (PPV) of 68.5% and 90.7% for clinician-collected and patient-collected specimens, respectively. Negative Predictive Values (NPV) of 99.6 % and 99.7% were obtained for clinician-collected and patient-collected specimens, respectively.

Table 42: *T. vaginalis* Performance by Specimen Collection Type and Specimen Collection Site

Site	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
Overall	**1438**	**9.9**	**96.5 (92.0-98.5) 137/142 [2]**	**95.1 (93.8-96.2) 1233/1296 [3]**	**1433**	**9.8**	**97.1 (92.9-98.9) 136/140 [4]**	**98.9 (98.2-99.4) 1279/1293 [5]**
#1	16	6.3	100 (20.7-100) 1/1	100 (79.6-100) 15/15	16	6.3	100 (20.7-100) 1/1	100 (79.6-100) 15/15
#2	1	0.0	NC	100 (20.7-100) 1/1	1	0.0	NC	100 (20.7-100) 1/1
#3	21	9.5	100 (34.2-100) 2/2	100 (83.2-100) 19/19	21	9.5	100 (34.2-100) 2/2	100 (83.2-100) 19/19
#4	213	17.4	97.3 (86.2-99.5) 36/37	83.5 (77.3-88.3) 147/176	211	17.1	100 (90.4-100) 36/36	98.9 (95.9-99.7) 173/175
#5	145	7.6	100 (74.1-100) 11/11	98.5 (94.7-99.6) 132/134	143	7.7	100 (74.1-100) 11/11	100 (97.2-100) 132/132
#6	68	1.5	100 (20.7-100) 1/1	98.5 (92.0-99.7) 66/67	68	1.5	100 (20.7-100) 1/1	100 (94.6-100) 67/67
#7	197	23.9	100 (92.4-100) 47/47	83.3 (76.6-88.4) 125/150	197	23.9	100 (92.4-100) 47/47	93.3 (88.2-96.3) 140/150
#8	1	100.0	100 (20.1-100) 1/1	NC	1	100.0	100 (20.1-100) 1/1	NC
#9	105	3.8	100 (51.0-100) 4/4	100 (96.3-100) 101/101	105	3.8	100 (51.0-100) 4/4	100 (96.3-100) 101/101
#10	17	0.0	NC	100 (81.6-100) 17/17	17	0.0	NC	100 (94.5-100) 66/66
#11	70	7.1	80.0 (37.6-96.4) 4/5	93.8 (85.2-97.6) 61/65	71	7.0	80.0 (37.6-96.4) 4/5	100 (92.2-99.7) 68/69
#12	130	3.1	75.0 (30.1-95.4) 3/4	100 (97.0-100) 126/126	129	3.1	75.0 (30.1-95.4) 3/4	100 (97.0-100) 125/125
#13	69	10.1	100 (64.6-100) 7/7	96.8 (89.0-99.1) 60/62	69	10.1	100 (64.6-100) 7/7	98.4 (91.4-99.7) 61/62
#14	8	0.0	NC	100 (67.6-100) 8/8	8	0.0	NC	100 (67.6-100) 8/8
#15	4	25.0	0.0 (0.0-79.3) 0/1	100 (43.9-100) 3/3	4	25.0	0.0 (0.0-79.3) 0/1	100 (43.9-100) 3/3
#16	28	10.7	100 (43.9-100) 3/3	100 (86.7-100) 25/25	28	10.7	100 (43.9-100) 3/3	100 (86.7-100) 71/72
#17	74	2.7	100 (34.2-100) 2/2	100 (94.9-100) 72/72	74	2.7	100 (34.2-100) 2/2	98.6 (92.5-99.8) 78/78
#18	83	4.8	100	100	83	4.8	100	100

			(51.0-100) 4/4	(95.4-100) 79/79			(51.0-100) 4/4	(95.4-100) 79/79
#19	71	4.2	66.7 (20.8-93.9) 2/3	100 (94.7-100) 68/68	71	4.2	66.7 (20.8-93.9) 2/3	100 (94.7-100) 68/68
#20	39	0.0	NC	100 (91.0-100) 39/39	39	0.0	NC	100 (91.0-100) 39/39
#21	78	11.5	100 (70.1-100) 9/9	100 (94.7-100) 69/69	77	10.4	100 (67.6-100) 8/8	100 (94.7-100) 69/69

NC = Not Calculated

[1] Score 95% Confidence Interval.

[2] Of the 5 samples with false negative results, 3 were negative with a second FDA-cleared TV molecular test.

[3] Of the 63 samples with false positive results, 56 were confirmed positive with a second FDA-cleared TV molecular test.

[4] Of the 4 samples with false negative results, 3 were negative with a second FDA-cleared TV molecular test.

[5] Of the 14 samples with false positive results, 8 were confirmed positive with a second FDA-cleared TV molecular test.

Tables 43, 44 and 45 below include *T. vaginalis* performance for clinician-collected and patient-collected vaginal specimens stratified respectively by age group, ethnicity, and patient clinical condition.

Table 43: *T. vaginalis* Performance by Age Group

Age Group	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	**Prevalence (%)**	**Sensitivity (95% CI) [1]**	**Specificity (95% CI) [1]**	**N**	**Prevalence (%)**	**Sensitivity (95% CI) [1]**	**Specificity (95% CI) [1]**
All	**1438**	**9.9**	**96.5 (92.0-98.5) 137/142**	**95.1 (93.8-96.2) 1233/1296**	**1433**	**9.8**	**97.1 (92.9-98.9) 136/140**	**98.9 (98.2-99.4) 1279/1293**
14-17	5	0.0	NC	100.0 (56.6-100.0) 5/5	5	0.0	NC	100.0 (56.6-100.0) 5/5
18-29	535	7.9	95.2 (84.2-98.7) 40/42	97.0 (95.0-98.1) 478/493	535	7.9	95.2 (84.2-98.7) 40/42	99.2 (97.9-99.7) 489/493
30-39	461	9.8	97.8 (88.4-99.6) 44/45	95.0 (92.4-96.7) 395/416	458	9.6	97.7 (88.2-99.6) 43/44	99.5 (98.3-99.9) 412/414
40-49	235	12.8	96.7 (83.3-99.4) 29/30	93.2 (88.9-95.9) 191/205	234	12.4	96.6 (82.8-99.4) 28/29	96.6 (93.1-98.3) 198/205
≥50	202	12.4	96.0 (80.5-99.3) 24/25	92.7 (87.8-95.7) 164/177	201	12.4	100.0 (86.7-100.0) 25/25	99.4 (96.9-99.9) 175/176

NC = Not Calculated

[1] Score 95% Confidence Interval.

Table 44: *T. vaginalis* Performance by Ethnicity

Ethnicity	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
All	**1438**	**9.9**	**96.5 (92.0-98.5) 137/142**	**95.1 (93.8-96.2) 1233/1296**	**1433**	**9.8**	**97.1 (92.9-98.9) 136/140**	**98.9 (98.2-99.4) 1279/1293**
Asian	67	6.0	100 (51.0-100) 4/4	98.4 (91.5-99.7) 62/63	66	6.1	100 (51.0-100) 4/4	100 (94.2-100) 62/62
Black/African-American	727	14.2	98.1 (93.2-99.5) 101/103	93.3 (91.0-95.0) 582/624	724	14.0	98.0 (93.1-99.5) 99/101	98.7 (97.5-99.3) 615/623
White (Hispanic/Latino)	257	6.6	94.1 (73.0-99.0) 16/17	95.0 (91.5-97.1) 228/240	258	6.6	94.1 (73.0-99.0) 16/17	97.9 (95.2-99.1) 236/241
White (Non Hispanic/Latino)	326	4.0	84.6 (57.8-95.7) 11/13	97.4 (95.0-98.7) 305/313	324	4.0	92.3 (66.7-98.6) 12/13	99.7 (98.2-99.9) 310/311
Other [2]	61	8.2	100 (56.6-100) 5/5	100 (93.6-100) 56/56	61	8.2	100 (56.6-100) 5/5	100 (93.6-100) 56/56

NC = Not Calculated

[1] Score 95% Confidence Interval.

[2] Includes patient-reported “other”, “mixed”, and unknown races.

Table 45: *T. vaginalis* Performance by Clinical Condition

Clinical Condition	Clinician-Collected Vaginal Swab				Patient-Collected Vaginal Swab			
	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]	N	Prevalence (%)	Sensitivity (95% CI) [1]	Specificity (95% CI) [1]
Use of antibiotics	5	0.0	NC	100 (56.6-100) 5/5	5	0.0	NC	100 (56.6-100) 5/5
Use of antifungals	7	0.0	NC	100 (64.6-100) 7/7	7	0.0	NC	100 (64.6-100) 7/7
Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2	2	0.0	NC	100 (34.2-100) 2/2
Recurrent symptoms of vaginitis in the last 12 months	841	8.1	95.6 (87.8-98.5) 65/68	94.7 (92.9-96.1) 732/773	839	8.0	97.0 (89.8-99.2) 65/67	98.4 (97.3-99.1) 760/772
Unprotected intercourse in the last 24 hours	94	12.8	91.7 (64.6-98.5) 11/12	96.3 (89.8-98.7) 79/82	93	12.9	100 (75.8-100) 12/12	100 (95.9-100) 81/81
Pregant	20	15.0	66.7 (20.8-93.9) 2/3	100 (81.6-100) 17/17	21	14.3	66.7 (20.8-93.9) 2/3	100 (82.4-100) 18/18
With menses	112	9.8	90.9 (62.3-98.4) 10/11	97.0 (91.6-99.0) 98/101	112	9.8	90.9 (62.3-98.4) 10/11	99.0 (94.6-99.8) 100/101
Without menses	1176	9.9	97.4 (92.7-99.1) 114/117	95.3 (93.8-96.4) 1009/1059	1173	9.8	97.4 (92.6-99.1) 112/115	98.9 (98.0-99.4) 1046/1058
Post-menopausal	150	9.3	92.9 (68.5-98.7) 13/14	92.6 (87.0-96.0) 126/136	148	9.5	100 (78.5-100) 14/14	99.3 (95.9-99.9) 133/134

NC = Not Calculated

[1] Score 95% Confidence Interval.

Multi-Analyte Detection Rates

The rates of multi-analyte detections by the Aptima CV/TV assay observed in the prospective clinical study are presented in Table 46 below. The most prevalent multi-analyte detection was a combination of C spp and *T. vaginalis* with 2.7% and 3.1% for clinician and patient-collected specimens, respectively. In total, 4.6% of clinician-collected and 5.1% of patient-collected specimens resulted in more than one Aptima CV/TV assay analyte reported.

Table 46: Aptima CV/TV Assay Multi-Analyte Detection Rates Observed During the Prospective Clinical Study

Analytes Detected	**Clinician-Collected Specimens (%)**	**Patient-Collected Specimens (%)**
C spp and *C. glabrata*	1.4 (21/1487)	1.6 (23/1478)
C spp and *T. vaginalis*	2.7 (40/1487)	3.1 (46/1478)
C spp, *C. glabrata*, and *T. vaginalis*	0.3 (4/1487)	0.3 (5/1478)
C. glabrata and *T. vaginalis*	0.2 (3/1487)	0.1 (1/1478)
Total	4.6 (68/1487)	5.1 (75/1478)

Note: Includes evaluable subjects with valid Aptima CV/TV assay results independent of whether reference results are available.

A comparison of multi-analyte detection based on all reportable specimen results is presented in Table 47 below for clinician and patient-collected specimens. Bolded entries represent multi-analyte detection events with concordant reference method and Aptima CV/TV assay results. Non-bolded entries represent specimens with discordant results. Concordant single detections are not represented.

Table 47: Total Number of Multi-Analyte Detections Observed in the Prospective Clinical Study - Concordant (Bolded Entries) and Discordant (Non-Bolded Entries) with Reference Methods

	Total Number of Occurrences (Clinician-Collected Specimens/Patient-Collected Specimens)								
	Reference Method								
	Organism Detections	C spp	C spp and *C.glabrata*	C spp and *T. vaginalis*	C spp, *C.glabrata*, and *T.*	*C.glabrata*	*C.glabrata* and *T. vaginalis*	*T. vaginalis*	Negative
Aptima CV/TV Assay	C spp		3/4	3/3	0/0	0/0	0/0	0/0	42/72
	C spp and *C.glabrata*	4/4	**14/15**	0/0	0/0	0/0	0/0	0/0	0/0
	C spp and *T. vaginalis*	0/1	2/0	**27/27**	2/1	0/0	1/1	7/16	1/0
	C spp, *C.glabrata*, and *T. vaginalis*	0/0	1/0	0/0	**3/4**	0/0	0/0	0/1	0/0
	C.glabrata	1/2	10/10	0/0	0/0		0/0	0/0	5/7
	C.glabrata and *T. vaginalis*	0/0	0/0	0/0	1/1	0/0	**0/0**	1/0	1/0
	T. vaginalis	0/1	0/0	1/1	0/0	0/0	0/0		58/12
	Negative	21/14	0/1	0/0	0/0	0/0	0/0	2/1	

Note: Includes evaluable subjects with valid Aptima CV/TV assay results and valid C spp, *C.glabrata*, and *T. vaginalis* reference results.

Contrived Clinical Specimens Testing

Due to anticipated low prevalence of *Candida glabrata*, a supplemental assessment of the Aptima CV/TV assay *C. glabrata* performance was also carried out testing contrived specimens. A total of 60 contrived *C. glabrata* positive samples and 60 true negative samples were tested with the Aptima CV/TV assay in this assessment to supplement the prospective clinical performance data.

Positive contrived samples were prepared by spiking lysates from five different *C. glabrata* strains into negative individual donor natural vaginal swab matrix (NVSM), at concentrations of 3×, 10×, and 20× the *C. glabrata* LoD. Negative samples were comprised of simulated vaginal swab matrix (SVSM) only.

Aptima CV/TV assay *C. glabrata* agreement with expected results was 100% across all contrived specimens. Study results are summarized in Table 48 below.

Table 48: Performance of the Aptima CV/TV Assay with *C. glabrata* Contrived Specimens

Specimen	**N**	**Aptima *C. glabrata* Positive**	**Aptima *C. glabrata* Negative**	**Positive Percent Agreement (95% CI)** [1]	**Negative Percent Agreement (95% CI)** [1]
Low Positive (3x LoD)	30	30	0	100% (88.6-100)	NC
Moderate Positive (10x LoD)	15	15	0	100% (79.6-100)	NC
High Positive (20x LoD)	15	15	0	100% (79.6-100)	NC
True Negative	60	0	60	NC	100% (94.0-100)

NC = Not Calculated

[1] Score 95% Confidence Interval.

Invalid Rates

A total of 3295 clinical specimens were processed in valid Aptima CV/TV assay runs during the clinical studies. Of these, 56 specimen had initial invalid results (1.7%, 95% CI: 1.3% - 2.2%). Upon retest, 16 specimens (0.5%, 95% CI: 0.3% - 0.8%) remained invalid.

Evaluation of the Aptima CV/TV Assay in Asymptomatic Women

Although the Aptima CV/TV assay is not intended for testing specimens from asymptomatic women, presence of *Candida* species and *C. glabrata* as colonizing normal flora has been reported in this population. The Aptima CV/TV assay was evaluated for detection of *Candida* species group (C spp) and *C. glabrata* with clinician-collected vaginal specimens collected from 171 asymptomatic women ≥ 14 years. Aptima CV/TV assay C spp and *C. glabrata* targets were detected with rates varying from 8.8% for *C. glabrata* to 21.1% for C spp. Results from the evaluation are presented in Table 49 below which also includes results for the most prevalent ethnic groups enrolled. C spp and *C. glabrata* were detected in all ethnic groups, except for Asian.

Table 49: Positivity of C spp and *C. glabrata* as Determined by the Aptima CV/TV Assay in Asymptomatic Women

Ethnicity	Clinician-Collected Vaginal Swab Specimens				
	Number of Vaginal Swab Specimens	Number of C spp Positives	C spp Positivity Rate	Number of *C. glabrata* Positives	*C. glabrata* Positivity Rate
All	171	36	21.1%	15	8.8%
Asian	5	0	0.0%	0	0.0%
Black/African-American	75	21	28.0%	9	12.0%
White (Hispanic/Latino)	41	7	17.1%	2	4.9%
White (Non Hispanic/Latino)	43	5	11.6%	3	7.0%
Other [1]	7	3	42.9%	1	14.3%

[1] Includes patient-reported "other", "mixed", and unknown races.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

In the Aptima CV/TV prospective clinical study, a total of 1485 clinician-collected and 1477 patient-collected vainal swab specimens were determined to be evaluable for C spp performance. The number and percentage of C spp positive cases per specified age group, as determined by the Aptima CV/TV assay for the clinician-collected and patient-collected vaginal specimens, are presented in Table 50 below.

Table 50: C spp Positives by the Aptima CV/TV Assay per Patient Age Group

Age Group	Clinician-Collected Vaginal Swab Specimens			Patient-Collected Vaginal Swab Specimens		
	Number of Vaginal Swab Specimens	Number of C spp Positives	C spp Positivity Rate	Number of Vaginal Swab Specimens	Number of C spp Positives	C spp Positivity Rate
14-17	5	2	40.0%	5	2	40.0%
18-29	549	183	33.3%	548	197	35.9%
30-39	475	150	31.6%	472	160	33.9%
40-49	246	66	26.8%	245	75	30.6%
≥50	210	42	20.0%	207	53	25.6%
Total	1485	443	29.8%	1477	487	33.0%

In the Aptima CV/TV prospective clinical study, a total of 1483 clinician-collected and 1475 patient-collected vainal swab specimens were determined to be evaluable for *C. glabrata* performance. The number and percentage of *C. glabrata* positive cases per specified age group, as determined by the Aptima CV/TV assay for the clinician-collected and patient-collected vaginal specimens, are presented in Table 51 below.

Table 51: *C. glabrata* Positives by the Aptima CV/TV Assay per Patient Age Group

Age Group	Clinician-Collected Vaginal Swab Specimens			Patient-Collected Vaginal Swab Specimens		
	Number of Vaginal Swab Specimens	Number of C spp Positives	C spp Positivity Rate	Number of Vaginal Swab Specimens	Number of C spp Positives	C spp Positivity Rate
14-17	5	0	0.0%	5	0	0.0%
18-29	549	13	2.4%	547	14	2.6%
30-39	473	13	2.7%	471	15	3.2%
40-49	246	17	6.9%	245	18	7.3%
≥50	210	20	9.5%	207	21	10.1%
Total	1483	63	4.2%	1475	68	4.6%

In the Aptima CV/TV prospective clinical, a total of 1438 clinician-collected and 1433 patient-collected vainal swab specimens were determined to be evaluable for *T. vaginalis* performance. The number and percentage of *T. vaginalis* positive cases per specified age group, as determined by the Aptima CV/TV assay for the clinician-collected and patient-collected vaginal specimens, are presented in Table 52 below.

Table 52: *T. vaginalis* Positives by the Aptima CV/TV Assay per Patient Age Group

Age Group	Clinician-Collected Vaginal Swab Specimens			Patient-Collected Vaginal Swab Specimens		
	Number of Vaginal Swab Specimens	Number of C spp Positives	C spp Positivity Rate	Number of Vaginal Swab Specimens	Number of C spp Positives	C spp Positivity Rate
14-17	5	0	0.0%	5	0	0.0%
18-29	535	55	10.3%	535	44	8.2%
30-39	461	65	14.1%	458	45	9.8%
40-49	235	43	18.3%	234	35	15.0%
≥50	202	37	18.3%	201	26	12.9%
Total	1438	200	13.9%	1433	150	10.5%

N. Instrument Name:

Panther System

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X_____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No __X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes _____X___ or No _____

3. Specimen Identification:

   By handheld barcode reader and positional checks.

4. Specimen Sampling and Handling:

   Fully automated.

5. Calibration:

   Hologic, Inc Field Service Engineers perform a luminometer calibration on the Panther System every 12 months as part of the Preventive Maintenance. Additionally, there are process controls and calibration checks on all of the dispensers, thermal devices, and the vacuum system.

6. Quality Control:

   In addition to the assay controls that are specific to the assay, the Panther System contains process controls that employ both hardware and software components. The process controls include, but are not limited to:
   - Verification that the sequence of assay processing steps is correct for each reaction.
   - Verification that the reaction incubation times and temperatures are correct.
   - Verification that reagents and fluids are appropriately dispensed.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.