

Aptima® BV Assay

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 For *in vitro* diagnostic use
 Rx only

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General Information

Intended Use

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

Summary and Explanation of the Test

Vaginitis syndrome is characterized by a spectrum of conditions: vaginal and vulvar irritation, odor, discharge, and pruritus (1). Causes of vaginitis include mechanical and chemical factors (feminine hygiene products, contraceptive materials, etc.) as well as infectious agents (1). Up to 90% of infectious vaginitis cases are caused by BV, vulvovaginal candidiasis (candida vaginitis, CV) and trichomoniasis (trichomonas vaginalis vaginitis, TV) (2). BV has been diagnosed in 22-50% of symptomatic patients, CV in 17-39%, and TV in 4-35% (1,2).

BV is responsible for the majority of infectious vaginitis cases. BV is characterized by a change in the vaginal microbiota dominated by *Lactobacillus* species to a polymicrobial anaerobe-dominated microbiota that includes *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella*, *Bacteroides*, *Peptostreptococcus*, *Mobiluncus*, *Sneathia* (*Leptotrichia*), *Mycoplasma*, and BV associated bacteria (3). This change in vaginal microbiota is associated with the onset of Amsel clinical signs, resulting from the biochemical and cytological changes in the vaginal milieu that are pathognomonic for BV (11). BV has been associated with pelvic inflammatory disease (4), cervicitis (5), elevated risk of acquisition of STIs, such as chlamydia, gonorrhea, HSV, HIV (6,7,8), spontaneous abortion, and preterm birth (9,10).

Diagnosis of BV based on clinical criteria (vaginal pH, presence of clue cells, whiff test, and discharge) has been proposed by Amsel (11). Nugent et al. proposed a classification for BV based on microscopic description of observed types of bacteria via Gram stain in vaginal swabs (12). Recent studies suggest that molecular diagnostic tools would be beneficial to improve diagnosis of BV and that nucleic acid amplification, targeting several BV-associated bacteria, could be utilized (13).

The Aptima BV assay is a real time TMA assay developed for use on the automated Panther system that detects and discriminates RNA markers from the *Lactobacillus* species group (*L. gasseri*, *L. crispatus* and *L. jensenii*), *Gardnerella vaginalis*, and *Atopobium vaginae* in clinician-collected and patient-collected vaginal swab specimens from symptomatic females. The Aptima BV assay uses an algorithm to report a qualitative result for BV based on detection of target organisms. The Aptima BV assay includes an internal control (IC).

Principles of the Procedure

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

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

Target amplification occurs via TMA, a transcription-based nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target RNA sequence, adding a promoter sequence for T7 RNA polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and hybridize specifically to the amplicon in real time. Each torch has a fluorophore and a quencher. The quencher suppresses the fluorescence of the fluorophore when the torch is not hybridized to the amplicon. When the torch binds to the amplicon, the fluorophore is separated from the quencher and emits a signal at a specific wavelength when excited by a light source. The Panther system detects and discriminates between four fluorescent signals corresponding to *Lactobacillus* group, *Atopobium vaginae*, *Gardnerella vaginalis*, and IC amplification products. The Panther system software compares signal emergence times for each target organism to calibration information to determine the BV Positive or Negative status of each sample.

Warnings and Precautions

- A. For *in vitro* diagnostic use.
- B. For professional use.
- C. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther/Panther Fusion System Operator's Manual* prior to performing this assay.
- D. Only personnel adequately trained in the use of the Aptima BV assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.
- E. For additional specific warnings and precautions, refer to the *Panther/Panther Fusion System Operator's Manual*.

Laboratory Related

- F. Use only supplied or specified disposable laboratory ware.
- G. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and

laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

- H. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Thoroughly clean and disinfect all work surfaces.
- I. Dispose of all materials that have come in contact with specimens and reagents in accordance with applicable national, international, and regional regulations (14, 15, 16). Thoroughly clean and disinfect all work surfaces.

Specimen Related

- J. Expiration dates for the collection kits pertain to the collection of specimens and not to the specimen testing. Samples collected any time prior to the expiration date of the collection kit, and transported and stored in accordance with the package insert, are valid for testing even if the expiration date on the collection tube has passed.
- K. Specimens may be infectious. Use Universal Precautions when performing this assay (14,15). Proper handling and disposal methods should be established according to local regulations (16). Only personnel adequately trained in the use of the Aptima BV assay and trained in handling infectious materials should perform this procedure.
- L. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.
- M. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over any open container. Change gloves if they come in contact with a specimen.
- N. Upon piercing, liquid can discharge from Aptima transfer tube caps under certain conditions. Refer to the *Panther System Test Procedure* for more information.
- O. If the lab receives an Aptima Multitest Swab Specimen Collection Kit transport tube with no swab, two swabs, a cleaning swab, or a swab not supplied by Hologic, the specimen must be rejected.

Assay Related

- P. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Controls, the calibrator, and assay fluids may be interchanged.
- Q. Cap and store reagents at the specified temperatures. The performance of the assay may be affected by use of improperly stored reagents. See *Reagent Storage and Handling Requirements* and *Panther System Test Procedure* for more information.
- R. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.
- S. Avoid microbial and nuclease contamination of reagents.

T. Do not use the reagent, control, or calibrator kits after the expiration date.

U. Some reagents used with the Aptima BV assay are labeled with risk and safety symbols.

Note: Hazard Communication information for labeling of globally marketed products reflects the US and EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologic.com/sds. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.

EU Hazard Information	
—	<p>Promoter Reagent MAGNESIUM CHLORIDE 35 - 40%</p> <p>H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment P280 - Wear eye protection/ face protection.</p>
—	<p>Target Capture Reagent HEPES 5 - 10% EDTA 1 - 5% LITHIUM HYDROXIDE, MONOHYDRATE 1 - 5%</p> <p>H412 - Harmful to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/ face protection</p>

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for the reagents, the calibrator, and the controls.

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2°C to 8°C		
Amplification Reconstitution Solution	15°C to 30°C	2°C to 8°C	30 days ¹
Enzyme Reagent	2°C to 8°C		
Enzyme Reconstitution Solution	15°C to 30°C	2°C to 8°C	30 days ¹
Promoter Reagent	2°C to 8°C		
Promoter Reconstitution Solution	15°C to 30°C	2°C to 8°C	30 days ¹
Target Capture Reagent	15°C to 30°C	15°C to 30°C ²	30 days ¹
Positive Calibrator	2°C to 8°C		Single use vial
Negative Control	2°C to 8°C		Single use vial
Positive Control	2°C to 8°C		Single use vial
Internal Control	2°C to 8°C		Single use vial

¹ When reagents are removed from the Panther system, they should be immediately returned to their appropriate storage temperatures.

² Storage condition for the working Target Capture Reagent (Target Capture Reagent with Internal Control added).

- B. Discard any unused reconstituted reagents and working Target Capture Reagent (wTCR) after 30 days or after the Master Lot expiration date, whichever comes first.
- C. Reagents stored on the Panther system have 120 hours of onboard stability. Reagents can be loaded onto the Panther system up to 8 times. The system logs each time the reagents are loaded.
- D. The Promoter Reagent and reconstituted Promoter Reagent are photosensitive. Protect these reagents from light during storage and preparation for use.
- E. Avoid cross-contamination during reagent handling and storage. Recap all reconstituted reagents with new reagent caps prior to storage.
- F. **Do not freeze reagents.**

Specimen Collection and Storage

Note: Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.

Note: Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.

Vaginal swab specimens can be tested with the Aptima BV assay. Assay performance has not been evaluated with specimens other than those collected with the following specimen collection kit:

- Aptima Multitest Swab Specimen Collection Kit

A. Specimen collection

Refer to the appropriate specimen collection kit package insert for specific collection instructions.

B. Specimen transport and storage before testing:

Only the following storage conditions should be used for specimens with the Aptima BV assay.

1. Swab specimens

- a. After collection, swab specimens in transport tubes can be stored at 2°C to 8°C for up to 30 days. If longer storage is needed, specimens may be stored at -20°C or -70°C for an additional 60 days.
- b. After collection, swab specimens in transport tubes can be stored at 15°C to 30°C for up to 30 days.

C. Specimen storage after testing:

- 1. Specimens that have been tested must be stored upright in a rack.
- 2. The specimen transport tubes should be covered with a new, clean plastic film or foil barrier.
- 3. If assayed samples need to be shipped, remove penetrable cap and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures should be maintained.

4. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 ± 100 relative centrifugal force (RCF) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination.**

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima BV assay are listed below for the Panther system. Reagent identification symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima BV Assay Kit

100 tests: 2 assay boxes, 1 calibrator kit, and 1 controls kit (Cat. No. PRD-05186)

Aptima BV Assay Refrigerated Box (store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	Amplification Reagent <i>Non-infectious nucleic acids dried in buffered solution.</i>	1 vial
E	Enzyme Reagent <i>Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.</i>	1 vial
PRO	Promoter Reagent <i>Non-infectious nucleic acids dried in buffered solution.</i>	1 vial
IC	Internal Control <i>Non-infectious RNA nucleic acids in buffered solution.</i>	1 x 0.3 mL

Aptima BV Assay Room Temperature Box (store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	Amplification Reconstitution Solution <i>Aqueous solution containing glycerol and preservatives.</i>	1 x 7.2 mL
ER	Enzyme Reconstitution Solution <i>HEPES buffered solution containing a surfactant and glycerol.</i>	1 x 5.8 mL
PROR	Promoter Reconstitution Solution <i>Aqueous solution containing glycerol and preservatives.</i>	1 x 4.5 mL
TCR	Target Capture Reagent <i>Buffered salt solution containing non-infectious nucleic acids and magnetic particles.</i>	1 x 26.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Aptima BV Assay Calibrator Kit (PRD-05188)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
PCAL	Positive Calibrator <i>Non-infectious nucleic acids in buffered solution.</i>	5 x 2.8 mL
	Calibrator Barcode Label	1 sheet

Aptima BV Assay Controls Kit (PRD-05187)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
CONTROL-	Negative Control <i>Non-infectious L. crispatus cultured cells in buffered solution.</i>	5 x 1.7 mL
CONTROL+	Positive Control <i>Non-infectious G. vaginalis and A. vaginae cultured cells in buffered solution.</i>	5 x 1.7 mL
	Control Barcode Label	1 sheet

Materials Required and Available Separately

Note: Materials available from Hologic have catalog numbers listed, unless otherwise specified.

Material	Cat. No.
Panther System	303095
Panther Run Kit for Real Time Assays (for real time assays only)	PRD-03455 (5000 tests)
<i>Aptima Assay Fluids Kit (also known as Universal Fluids Kit)</i> <i>Contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent</i>	303014 (1000 tests)
<i>Multi-tube units (MTUs)</i>	104772-02
<i>Panther Waste Bag Kit</i>	902731
<i>Panther Waste Bin Cover</i>	504405
Or, Panther System Run Kit	303096 (5000 tests)
<i>When running non-real time-TMA assays in parallel with real time-TMA assays</i> <i>Contains MTUs, waste bags, waste bin covers, auto detect, and assay fluids</i>	
Aptima Assay Fluids Kit	303014 (1000 tests)
<i>Contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent</i>	
Multi-tube units (MTUs)	104772-02

Material	Cat. No.
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable. <i>Not all products are available in all regions. Contact your representative for region-specific information</i>	901121(10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Bleach, 5.0% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	--
Disposable, powderless gloves	--
Aptima penetrable caps	105668
Replacement non-penetrable caps	103036A
Reagent Replacement Caps	
<i>Amplification, Enzyme, and Promoter reagent reconstitution bottles</i>	CL0041 (100 caps)
<i>TCR bottle</i>	501604 (100 caps)
Plastic-backed laboratory bench covers	--
Lint-free wipes	--
Pipettor	--
Tips	--
Tube Rocker	--

Panther System Test Procedure

Note: See the Panther/Panther Fusion System Operator's Manual for additional Panther system procedural information.

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (Step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (Step A.1).

B. Reagent Reconstitution/Preparation of a New Kit

Note: Reagent reconstitution should be performed prior to beginning any work on the Panther system.

1. Prior to testing, Amplification, Enzyme, and Promoter Reagents must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate reconstitution solution.
 - a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.
 - b. Pair each reconstitution solution with its lyophilized reagent. Before attaching the reconstitution collar, ensure that the reconstitution solution and reagent have matching label symbols.

- c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
- d. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
- e. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
- f. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
- g. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
- h. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (Figure 1, Step 4).
- i. Wait at least 15 minutes for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.
- j. Remove the reconstitution collar and glass vial (Figure 1, Step 6).
- k. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).
- l. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

Option: Additional mixing of the Amplification, Enzyme, and Promoter Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

Warning: Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Panther system.

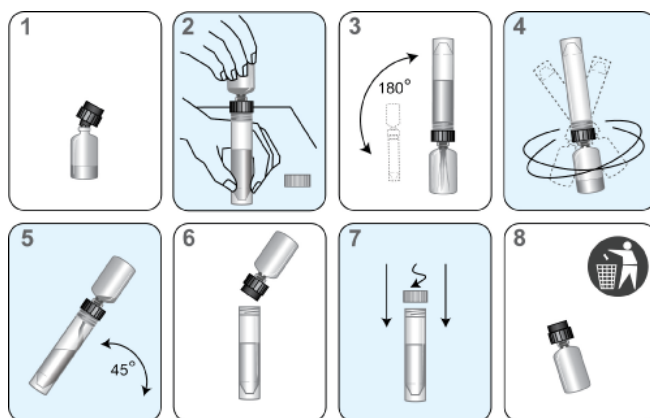


Figure 1. Reagent Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)
 - a. Pair the appropriate bottles of TCR and IC.
 - b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
 - c. Open the bottle of TCR, and set the cap on a clean, covered work surface.

- d. Open the bottle of IC and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
- e. Cap the bottle and gently swirl the solution to mix the contents. Avoid creating foam during this step.
- f. Record operator initials and the current date on the label.
- g. Discard the IC bottle and cap.

C. Reagent Preparation for Previously Prepared Reagents

1. Previously prepared Amplification, Enzyme, and Promoter reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

Option: The reagents may be brought to room temperature by placing the reconstituted Amplification, Enzyme, and Promoter Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

2. If wTCR contains precipitate, warm wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
3. Verify that the reagents have not exceeded their storage stability times, including onboard stability.
4. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam when inverting reagents.
5. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

D. Specimen Handling

1. Allow the specimens to reach room temperature prior to processing.
2. **Do not vortex specimens.**
3. Visually confirm that each specimen tube meets the following criteria:
 - a. The presence of a single pink Aptima collection swab in a swab specimen transport tube.
4. Inspect specimen tubes before loading into rack:
 - a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
 - b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.

Note: Failure to follow Steps 4a-4b may result in liquid discharge from the specimen tube cap.

Note: Up to 4 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 4 aliquots from the specimen tube can lead to processing errors.

E. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes*. Make sure that the appropriately sized reagent racks and TCR adapters are used.

Procedural Notes

A. Calibrator and Controls

Allow the calibrator and controls to reach room temperature prior to processing.

1. The positive calibrator, positive control and negative control tubes can be loaded in any rack position or in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following 2 conditions has been met:
 - a. The calibrator and controls are currently being processed by the system.
 - b. Valid results for the calibrator and controls are registered on the system.
2. Once the calibrator and control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be tested with the associated kit up to 24 hours **unless**:
 - a. The calibrator result or control results are invalid.
 - b. The associated assay reagent kit is removed from the system.
 - c. The associated assay reagent kit has exceeded stability limits.
3. Each calibrator or each control tube can be used once. Attempts to use more than once can lead to processing errors.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control

An operator may 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.

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 the 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

An IC is added to each sample with the wTCR. 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/Panther Fusion System Operator's Manual*.

Test Interpretation

Test results are automatically determined by the assay software. The table below shows the possible results reported in a valid run and result interpretations. Samples with invalid test results should be retested.

Table 1: Result Interpretation

BV Result	Interpretation
Positive	Positive for BV

Table 1: Result Interpretation

BV Result	Interpretation
Negative	Negative for BV
Invalid	Invalid test

Limitations

- A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.
- B. The effects of other potential variables such as vaginal discharge, use of tampons, and specimen collection variables have not been determined.
- C. Performance with specimen types other than vaginal swab specimens has not been evaluated.
- D. Reliable results are dependent on adequate specimen collection, transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. See *Specimen Collection and Storage* for instructions. For detailed information, refer to the appropriate instructions for use.
- E. Therapeutic failure or success cannot be determined with the Aptima BV assay since nucleic acid may persist following appropriate antimicrobial therapy.
- F. Bacterial species targeted by the Aptima BV assay may comprise part of the normal microbiome for a significant number of women; a BV positive result should be interpreted in conjunction with other clinical data available to the clinician.
- G. A negative result does not preclude a possible infection. Test results may be affected by improper specimen collection, technical error, or specimen mix-up.
- H. The Aptima BV assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the number of organisms in a specimen.
- I. Performance of the assay has not been evaluated in individuals less than 14 years of age.
- J. Customers must independently validate an LIS transfer process.
- K. The Aptima BV assay has not been evaluated for use with specimens collected by patients at home.
- L. Collection and testing of patient-collected vaginal swab specimens with the Aptima BV assay is not intended to replace clinical examination.
- M. Public health recommendations should be consulted regarding testing for additional sexually transmitted infections (STI) for patients with a positive result with the Aptima BV assay.

- N. Additional microorganisms not detected by the Aptima BV assay such as *Prevotella* species and *Mobiluncus* species, *Ureaplasma*, *Mycoplasma*, and numerous fastidious or uncultivated anaerobes have also been found in women with BV, but are less associated with BV due to their relatively low prevalence, sensitivity, and/or specificity (17).
- O. Interference with the Aptima BV assay was observed in the presence of the following substances: Mucus (1.5% V/V), Vaginal Moisturizing Gel (0.5% W/V) and Tioconazole (5% W/V).
- P. Cross-reactivity was observed with the Aptima BV assay in the presence of *Lactobacillus acidophilus* (1×10^4 CFU/mL).
- Q. A positive test result does not necessarily indicate the presence of viable organisms. A positive result is indicative of the presence of target RNA.

Panther System Expected Values

The prevalence of bacterial vaginosis in patient populations depends on age, race/ethnicity, risk factors, the type of clinic, and the sensitivity of the test used to detect infections. A summary of the BV positivity in symptomatic subjects, as determined by the Aptima BV assay on the Panther system, is shown in Table 2 for the multi-center study, by clinical site and overall.

Table 2: Positivity as Determined by the Aptima BV Assay in Symptomatic Women by Specimen Type and Clinical Site

%Positivity (# positive/# tested with valid results)		
Site	Clinician-collected Vaginal Swabs	Patient-collected Vaginal Swabs
1	40.0 (6/15)	46.7 (7/15)
2	20.0 (1/5)	0.0 (0/5)
3	63.6 (14/22)	63.6 (14/22)
4	51.9 (108/208)	60.5 (124/205)
5	48.5 (64/132)	50.8 (66/130)
6	46.5 (33/71)	50.7 (36/71)
7	68.1 (130/191)	69.3 (131/189)
8	100.0 (1/1)	100.0 (1/1)
9	48.0 (49/102)	54.9 (56/102)
10	70.6 (12/17)	70.6 (12/17)
11	50.7 (34/67)	50.7 (34/67)
12	32.8 (41/125)	34.1 (42/123)
13	63.2 (43/68)	62.3 (43/69)
14	55.6 (5/9)	55.6 (5/9)
15	50.0 (2/4)	50.0 (2/4)
16	58.6 (17/29)	65.5 (19/29)
17	49.4 (39/79)	51.3 (41/80)
18	64.4 (56/87)	64.4 (56/87)
19	45.6 (31/68)	50.0 (34/68)
20	11.1 (4/36)	19.4 (7/36)
21	58.4 (45/77)	57.9 (44/76)
All	52.0 (735/1413)	55.1 (774/1405)

Panther System Assay Performance

Reproducibility

Aptima BV assay reproducibility was evaluated on the Panther system at three US sites using seven panel members. Two operators performed testing at each site. Each operator performed one run per day over six days using one reagent lot over the course of testing. Each run had three replicates of each panel member.

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

The agreement with expected results was 100% for all panel members.

Signal variability of the Aptima BV assay was calculated for each target in analyte positive panel members. Only samples with valid results were included in the analyses. Variability, calculated between sites, between operators, between days, between runs, within run, and overall, is shown in Table 3 to Table 5 for *Lactobacillus*, *G. vaginalis*, and *A. vaginae* positive panel members, respectively.

Table 3: Signal Variability for *Lactobacillus* Positive Panel Members

Panel Description	N	Mean TTime ¹	Between Sites		Between Operators		Between Days		Between Runs		Within Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
<i>L. crispatus</i> BV Negative ²	108	19.73	0.30	1.53	0.61	3.07	0.13	0.64	0.63	3.17	0.12	0.62	0.94	4.76
<i>L. jensenii</i> BV Low Positive ²	108	24.31	0.00	0.00	0.77	3.16	0.00	0.00	0.80	3.28	0.15	0.62	1.12	4.60

CV = coefficient of variation, SD = standard deviation.

¹ TTime is shown for *Lactobacillus* only.

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

Note: In the event that variability from some factors is numerically negative, SD and CV are shown as 0.00.

Table 4: Signal Variability for *G. vaginalis* Positive Panel Members

Panel Description	N	Mean TTime ¹	Between Sites		Between Operators		Between Days		Between Runs		Within Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
<i>G. vaginalis</i> Low Positive	108	15.69	0.35	2.26	0.40	2.52	0.00	0.00	0.38	2.43	0.15	0.96	0.67	4.28
<i>G. vaginalis</i> Moderate Positive	108	14.33	0.30	2.07	0.37	2.58	0.00	0.00	0.35	2.41	0.14	0.98	0.60	4.21

CV = coefficient of variation, SD = standard deviation.

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

Note: In the event that variability from some factors is numerically negative, SD and CV are shown as 0.00.

Table 5: Signal Variability for *A. vaginae* Positive Panel Members

Panel Description	N	Mean TTime ¹	Between Sites		Between Operators		Between Days		Between Runs		Within Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
<i>A. vaginae</i> BV Negative ²	108	18.01	0.39	2.15	0.44	2.46	0.08	0.45	0.47	2.59	0.18	0.97	0.78	4.30
<i>A. vaginae</i> Low Positive	108	14.95	0.38	2.52	0.41	2.75	0.00	0.00	0.39	2.61	0.14	0.93	0.69	4.64
<i>A. vaginae</i> BV Low Positive ²	108	14.94	0.41	2.76	0.37	2.51	0.00	0.00	0.37	2.45	0.17	1.13	0.69	4.60
<i>A. vaginae</i> Moderate Positive	108	13.99	0.29	2.08	0.36	2.60	0.03	0.18	0.39	2.82	0.14	1.00	0.63	4.48

CV = coefficient of variation, SD = standard deviation.

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

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

Note: In the event that variability from some factors is numerically negative, SD and CV are shown as 0.00.

Panther System Clinical Performance

Performance Characteristics in Symptomatic Subjects

A prospective, multi-center clinical study was conducted to establish the clinical performance characteristics of the Aptima BV assay on the Panther system. Female subjects 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, STI, medical group clinics, and clinical research centers.

Three (3) vaginal swab samples were collected from each subject: one clinician-collected swab sample and one patient-collected swab sample were collected using the Aptima Multitest Swab Specimen Collection Kit for Aptima BV assay testing, and one clinician-collected swab sample was collected for reference method testing. Aptima samples were tested with the Aptima BV assay on the Panther system at three sites. BV infection status was determined using a combination of Nugent interpretations and Amsel criteria from the final vaginal swab sample.

- Samples with normal flora as per the Nugent interpretation were considered negative; samples positive for BV flora were considered positive.
- Samples with intermediate Nugent interpretations were classified as positive or negative for BV using modified Amsel criteria. Samples positive for $\geq 20\%$ clue cells and at least 1 of the 2 following criteria were considered Amsel positive: vaginal pH > 4.5 and positive whiff test.
- Samples that were unable to be assessed for the Nugent criteria, and samples with indeterminate Nugent interpretation for which a modified Amsel result was not available, were considered to have unknown BV infection status.

Performance characteristics for each sample, with corresponding 2-sided 95% Score confidence intervals (CIs), were estimated relative to the BV infection status.

Of the 1519 symptomatic subjects enrolled, 102 were not evaluable due to withdrawal ($n = 17$) or unknown BV infection status ($n = 85$). The remaining 1417 subjects were evaluable for at least one of the sample types. Table 6 shows the demographics of evaluable subjects.

Table 6: Demographics of Evaluable Subjects

Characteristics		Total
Total, N	N	1417
Age (years)	Mean ± SD	34.7 ± 11.11
	Median	33.0
	Range	14-75
Age category (years), n (%)	14-17	4 (0.3)
	18-29	537 (37.9)
	30-39	469 (33.1)
	40-49	235 (16.6)
	>50	172 (12.1)
Race/Ethnicity, n (%)	Asian	67 (4.7)
	Black or African American	731 (51.6)
	White (Hispanic or Latino)	248 (17.5)
	White (Not Hispanic or Latino)	307 (21.7)
	Other ¹	64 (4.5)

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

For the 1417 evaluable subjects, 1413 clinician-collected vaginal swab samples and 1405 patient-collected vaginal swab samples were included in the analyses. The sensitivity and specificity of the Aptima BV assay for the detection of BV are shown for both sample types overall and by site in Table 7. Assay performance is shown stratified by race/ethnicity in Table 8, and by clinical condition in Table 9.

Table 7: Performance Characteristics by Collection Site in Symptomatic Women

Site	Clinician-collected Vaginal Swabs				Patient-collected Vaginal Swabs			
	N	Prev (%)	Sensitivity % (95% CI) ¹	Specificity % (95% CI) ¹	N	Prev (%)	Sensitivity % (95% CI) ¹	Specificity % (95% CI) ¹
All	1413	49.2	95.0 (93.1-96.4) 660/695²	89.6 (87.1-91.6) 643/718³	1405	49.3	97.3 (95.8-98.2) 673/692⁴	85.8 (83.1-88.2) 612/713⁵
1	15	40.0	100 (61.0-100) 6/6	100 (70.1-100) 9/9	15	40.0	100 (61.0-100) 6/6	88.9 (56.5-98.0) 8/9
2	5	20.0	100 (20.7-100) 1/1	100 (51.0-100) 4/4	5	20.0	0.0 (0.0-79.3) 0/1	100 (51.0-100) 4/4
3	22	59.1	100 (77.2-100) 13/13	88.9 (56.5-98.0) 8/9	22	59.1	100 (77.2-100) 13/13	88.9 (56.5-98.0) 8/9
4	208	53.4	89.2 (82.0-93.7) 99/111	90.7 (83.3-95.0) 88/97	205	53.7	96.4 (91.0-98.6) 106/110	81.1 (72.0-87.7) 77/95
5	132	39.4	96.2 (87.0-98.9) 50/52	82.5 (72.7-89.3) 66/80	130	40.0	98.1 (89.9-99.7) 51/52	80.8 (70.7-88.0) 63/78
6	71	45.1	90.6 (75.8-96.8) 29/32	89.7 (76.4-95.9) 35/39	71	45.1	100 (89.3-100) 32/32	89.7 (76.4-95.9) 35/39
7	191	66.0	97.6 (93.2-99.2) 123/126	89.2 (79.4-94.7) 58/65	189	65.6	98.4 (94.3-99.6) 122/124	86.2 (75.7-92.5) 56/65
8	1	100.0	100 (20.7-100) 1/1	NC	1	100.0	100 (20.7-100) 1/1	NC
9	102	48.0	87.8 (75.8-94.3) 43/49	88.7 (77.4-94.7) 47/53	102	48.0	95.9 (86.3-98.9) 47/49	83.0 (70.8-90.8) 44/53
10	17	76.5	92.3 (66.7-98.6) 12/13	100 (51.0-100) 4/4	17	76.5	92.3 (66.7-98.6) 12/13	100 (51.0-100) 4/4
11	67	46.3	96.8 (83.8-99.4) 30/31	88.9 (74.7-95.6) 32/36	67	46.3	96.8 (83.8-99.4) 30/31	88.9 (74.7-95.6) 32/36
12	125	28.0	94.3 (81.4-98.4) 33/35	91.1 (83.4-95.4) 82/90	123	29.3	91.7 (78.2-97.1) 33/36	89.7 (81.5-94.5) 78/87
13	68	55.9	100 (90.8-100) 38/38	83.3 (66.4-92.7) 25/30	69	55.1	97.4 (86.5-99.5) 37/38	80.6 (63.7-90.8) 25/31
14	9	44.4	100 (51.0-100) 4/4	80.0 (37.6-96.4) 4/5	9	44.4	100 (51.0-100) 4/4	80.0 (37.6-96.4) 4/5
15	4	25.0	100 (20.7-100) 1/1	66.7 (20.8-93.9) 2/3	4	25.0	100 (20.7-100) 1/1	66.7 (20.8-93.9) 2/3
16	29	55.2	93.8 (71.7-98.9) 15/16	84.6 (57.8-95.7) 11/13	29	55.2	100 (80.6-100) 16/16	76.9 (49.7-91.8) 10/13

Table 7: Performance Characteristics by Collection Site in Symptomatic Women (continued)

Site	Clinician-collected Vaginal Swabs				Patient-collected Vaginal Swabs			
	N	Prev (%)	Sensitivity % (95% CI) ¹	Specificity % (95% CI) ¹	N	Prev (%)	Sensitivity % (95% CI) ¹	Specificity % (95% CI) ¹
17	79	45.6	97.2 (85.8-99.5) 35/36	90.7 (78.4-96.3) 39/43	80	45.0	100 (90.4-100) 36/36	88.6 (76.0-95.0) 39/44
18	87	60.9	98.1 (90.1-99.7) 52/53	88.2 (73.4-95.3) 30/34	87	60.9	100 (93.2-100) 53/53	91.2 (77.0-97.0) 31/34
19	68	42.6	100 (88.3-100) 29/29	94.9 (83.1-98.6) 37/39	68	42.6	100 (88.3-100) 29/29	87.2 (73.3-94.4) 34/39
20	36	16.7	66.7 (30.0-90.3) 4/6	100 (88.6-100) 30/30	36	16.7	66.7 (30.0-90.3) 4/6	90.0 (74.4-96.5) 27/30
21	77	54.5	100 (91.6-100) 42/42	91.4 (77.6-97.0) 32/35	76	53.9	97.6 (87.4-99.6) 40/41	88.6 (74.0-95.5) 31/35

CI = confidence interval, NC = not calculable, Prev = prevalence.

¹ Score CI.

² Of the 35 false negatives results, 10 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 15 were negative by Amsel.

³ Of the 75 false positive results, 46 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 6 were positive by Amsel.

⁴ Of the 19 false negative results, 6 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 7 were negative by Amsel.

⁵ Of the 101 false positive results, 55 subjects were Nugent intermediates and had BV infection status determined by Amsel criteria, and 9 were positive by Amsel.

Table 8: Performance Characteristics by Race/Ethnicity in Symptomatic Women

Specimen Type	Race/Ethnicity	N	Prev (%)	Sensitivity % (95% CI) ¹	Specificity % (95% CI) ¹
Clinician-collected Vaginal Swabs	All	1413	49.2	95.0 (93.1-96.4) 660/695	89.6 (87.1-91.6) 643/718
	Asian	67	31.3	95.2 (77.3-99.2) 20/21	91.3 (79.7-96.6) 42/46
	Black/African-American	729	61.0	95.5 (93.2-97.1) 425/445	89.1 (84.9-92.2) 253/284
	White (Hispanic/Latino)	247	46.2	96.5 (91.3-98.6) 110/114	86.5 (79.6-91.3) 115/133
	White (Not Hispanic/Latino)	306	28.8	88.6 (80.3-93.7) 78/88	91.7 (87.3-94.7) 200/218
	Other ²	64	42.2	100 (87.5-100) 27/27	89.2 (75.3-95.7) 33/37
Patient-collected Vaginal Swabs	All	1405	49.3	97.3 (95.8-98.2) 673/692	85.8 (83.1-88.2) 612/713
	Asian	65	30.8	95.0 (76.4-99.1) 19/20	86.7 (73.8-93.7) 39/45
	Black/African-American	727	61.2	97.5 (95.6-98.6) 434/445	84.8 (80.1-88.5) 239/282
	White (Hispanic/Latino)	246	45.9	99.1 (95.2-99.8) 112/113	83.5 (76.2-88.8) 111/133
	White (Not Hispanic/Latino)	303	28.7	93.1 (85.8-96.8) 81/87	87.5 (82.4-91.3) 189/216
	Other ²	64	42.2	100 (87.5-100) 27/27	91.9 (78.7-97.2) 34/37

CI = confidence interval, Prev = prevalence

¹ Score CI.² Includes patient-reported other, mixed, and unknown races.

Table 9: Performance Characteristics by Clinical Condition in Symptomatic Women

Collection Type	Clinical Condition	N ¹	Prev (%)	Sensitivity % (95% CI) ²	Specificity % (95% CI) ²
Clinician-collected Vaginal Swabs	All	1413	49.2	95.0 (93.1-96.4) 660/695	89.6 (87.1-91.6) 643/718
	Use of antibiotics	3	33.3	100 (20.7-100) 1/1	100 (34.2-100) 2/2
	Use of antifungals	8	25.0	100 (34.2-100) 2/2	100 (61.0-100) 6/6
	Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2
	Recurrent symptoms of vaginitis in the last 12 months	832	49.8	95.2 (92.7-96.9) 394/414	88.8 (85.4-91.4) 371/418
	Unprotected intercourse in the last 24 hours	94	57.4	92.6 (82.4-97.1) 50/54	85.0 (70.9-92.9) 34/40
	Pregnant	20	45.0	100 (70.1-100) 9/9	100 (74.1-100) 11/11
	With Menses	111	46.8	96.2 (87.0-98.9) 50/52	86.4 (75.5-93.0) 51/59
	Without Menses	1177	50.6	95.6 (93.7-97.0) 569/595	89.3 (86.6-91.6) 520/586
	Post-menopausal	125	38.4	85.4 (72.8-92.8) 41/48	93.5 (85.7-97.2) 72/77
Patient-collected Vaginal Swabs	All	1405	49.3	97.3 (95.8-98.2) 673/692	85.8 (83.1-88.2) 612/713
	Use of antibiotics	3	33.3	100 (20.7-100) 1/1	100 (34.2-100) 2/2
	Use of antifungals	8	25.0	100 (34.2-100) 2/2	100 (61.0-100) 6/6
	Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2
	Recurrent symptoms of vaginitis in the last 12 months	828	49.9	98.1 (96.2-99.0) 405/413	85.1 (81.3-88.2) 353/415
	Unprotected intercourse in the last 24 hours	94	57.4	98.1 (90.2-99.7) 53/54	75.0 (59.8-85.8) 30/40
	Pregnant	20	45.0	100 (70.1-100) 9/9	90.9 (62.3-98.4) 10/11
	With Menses	109	47.7	100 (93.1-100) 52/52	84.2 (72.6-91.5) 48/57
	Without Menses	1175	50.6	97.5 (95.9-98.5) 579/594	85.4 (82.3-88.0) 496/581
	Post-menopausal	121	38.0	91.3 (79.7-96.6) 41/46	90.7 (82.0-95.4) 68/75

CI = confidence interval, NC = not calculable, Prev = prevalence

¹ Subjects may report multiple clinical conditions; sum of subject numbers in all subgroups does not equal the total number of subjects.

² Score CI.

Positivity Rates in Asymptomatic Women

The detection of an imbalance in the vaginal microbiome is relevant for treatment decisions. Although the Aptima BV assay is not intended for use in testing samples from asymptomatic women, organisms associated with BV infection and detected by the Aptima BV assay also may be present in asymptomatic women. Presence of the Aptima BV assay bacterial targets was assessed in clinician-collected vaginal swab samples from 172 asymptomatic women. A summary of the BV detection rates, as determined by the Aptima BV assay, is shown in Table 10 for the multi-center study overall and by race/ethnicity.

Table 10: Positivity as Determined by the Aptima BV Assay in Asymptomatic Women

Race/Ethnicity	% Positivity (# positive/# tested with valid results)
All	40.7% (70/172)
Asian	40.0% (2/5)
Black/African American	52.0% (39/75)
White (Hispanic/Latino)	43.9% (18/41)
White (Not Hispanic/Latino)	15.9% (7/44)
Other ¹	57.1% (4/7)

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

Invalid Rates

A total of 3175 clinician- and patient-collected samples from symptomatic and asymptomatic subjects were processed in valid Aptima BV runs to establish clinical performance. Of these, 0.7% had initial invalid results. Upon retest, 0.1% remained invalid and were excluded from all analyses.

Panther System Analytical Performance

Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LoD) and BV positivity limits of the Aptima BV assay were determined by testing a series of panels consisting of *L. crispatus*, *L. gasseri*, *L. jensenii*, *G. vaginalis*, or *A. vaginae* cell lysates diluted into simulated vaginal swab matrix (SVSM). A minimum of 20 replicates of each panel member were tested with each of two reagent lots for a minimum of 40 replicates per panel member. The predicted detection limits for each organism calculated using Probit analysis are shown in Table 11.

Table 11: Limit of Detection of the Aptima BV Assay

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

¹ Predicted BV Positivity Limits (C₉₅) for *A. vaginae* and *G. vaginalis* in the Aptima BV assay are approximately 5.10 log CFU/mL and 4.86 log CFU/mL, respectively.

Analytical Inclusivity

Five strains of each target organism were tested using lysate targeting 3X C₉₅ for *G. vaginalis* and *A. vaginae*, and 3X LoD for Lactobacillus species (*L. crispatus*, *L. gasseri*, and *L. jensenii*) in SVSM. The Aptima BV Assay was BV positive for all five strains of *G. vaginalis* and *A. vaginae* at 3X C₉₅. All five strains of *L. crispatus* and *L. gasseri* were detected at 3X LoD. Three of the five strains of *L. jensenii* were detected at 3X LoD, and the remaining two strains at 10X LoD.

Cross-Reactivity and Microbial Interference

Cross-reactivity and microbial interference with the Aptima BV assay were evaluated in the presence of non-targeted organisms. A panel consisting of 62 organisms (Table 12) was tested in SVSM in the absence or in the presence of *L. crispatus* at 3X LoD, *G. vaginalis* at 3X C₉₅, or *A. vaginae* at 3X C₉₅. No cross-reactivity or microbial interference was observed for any of the 62 organisms tested in the Aptima BV assay at the concentrations listed in Table 12.

Table 12: Cross-Reactivity and Microbial Interference Panel

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

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

¹ In Vitro Transcript tested.

² *Lactobacillus acidophilus* affects BV positivity at 1x10⁴ CFU/mL or higher.

Interference

Potentially interfering substances were tested in the Aptima BV assay. Panels were built in SVSM and evaluated for potential effects on assay sensitivity and specificity. Sensitivity performance was evaluated separately for *L. crispatus* by spiking lysate at 3X LoD, and for *G. vaginalis* and *A. vaginae* by spiking lysate at 3X C₉₅. Negative panels containing each substance were also evaluated for specificity.

No interference was observed in the presence of the following exogenous and endogenous substances tested at the concentrations listed in Table 13.

Table 13: Interfering Substances Panel

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

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

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

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

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

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

Within Laboratory Precision

Within Lab Precision was evaluated on three Panther systems at one site. Three operators performed testing across 21 days and three reagent lots. Each operator performed two runs per day using an 11 member panel. Each run consisted of three replicates of each panel member.

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

BV percent positive results for each panel are presented in Table 14. Signal variability (TTime) of the Aptima BV assay was calculated for each target in analyte positive panel members. Variability calculated between operators, between instruments, between days, between lots, between runs, within run, and overall, is shown in Table 15 through Table 17.

Table 14: BV Positivity of Precision Panels

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

¹ Three invalid results were excluded from the analysis.

Table 15: Signal Variability of *Lactobacillus* Panel Members

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

CV = Coefficient of variation.

¹ TTime is shown for *Lactobacillus* only.

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

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

⁴ Three invalid results were excluded from the analysis.

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.00.

Table 16: Signal Variability of *G. vaginalis* Panel Members

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

CV = Coefficient of variation, Mod = moderate.

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

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

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

⁴ Three invalid results were excluded from the analysis.

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.00.

Table 17: Signal Variability of *A. vaginae* Panel Members

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

CV = Coefficient of variation, Mod = moderate

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

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

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

⁴ Three invalid results were excluded from the analysis.

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.00.

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Revision History	Date	Description
AW-23712 Rev. 001	August 2022	<ul style="list-style-type: none"> • Created Aptima Bacterial Vaginosis (BV) assay IFU AW-23712 Rev. 001 based on AW-18811 Rev. 003 for regulatory compliance with IVDR. • Updated Hazard information. • Updated contact information including: EC Rep, CE Mark, Australian Rep information, and technical support. • Miscellaneous style and formatting updates.