Aptima® CV/TV Assay

Instructions for Use
For *in vitro* diagnostic use
Rx only

General Information

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) technology to detect and qualitatively report results for the following organisms:

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

The assay differentiates between *C. 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 TV, the assay targets ribosomal RNA (rRNA) and differentiates the result from results for *C. 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.

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 bacterial vaginosis (BV), vulvovaginal candidiasis (candida vaginitis, CV) and trichomoniasis (TV) (2). BV has been diagnosed in 22-50% of symptomatic patients, CV in 17-39%, and TV in 4-35% (1, 2).

CV, commonly known as a yeast infection, is the second and most frequent cause of vaginitis. CV is characterized by an overgrowth of *Candida* species in the vaginal tract and is associated with clinical signs of inflammation (3). Up to 89% of CV cases are caused by *C. albicans*, while non-albicans species may be responsible for 11% (3). Characteristic symptoms for CV include abnormal vaginal discharge, vaginal soreness, pruritus, dyspareunia, and external dysuria (4). *C. glabrata*, which is responsible for the majority of non-albicans CV in the U.S., may have decreased susceptibility to standard antimycotic therapeutic intervention compared to *C. albicans* (4, 5). *C. glabrata* infections therefore require special attention in clinical management.

TV is the third most common cause of infectious vaginitis (2). The causative agent, the protozoan parasite TV, is transmitted by unprotected penile-vaginal sex (4). Women infected with TV during pregnancy have increased risk for adverse pregnancy outcomes, such as premature rupture of membranes, preterm delivery, and low birth weight (4). TV infection is associated with an increased risk of HIV acquisition and transmission (6, 7), as well as prolonged HPV infection (7) and concurrent sexually transmitted infections (chlamydia, gonorrhea, and herpes simplex virus types 1 & 2) (8).

CV and TV may be detected by microscopy, culture, and nucleic acid using specimens collected with vaginal swabs.

The Aptima CV/TV assay is a real time TMA assay developed for use on the automated Panther system that detects and discriminates RNA markers from C spp, *C. glabrata*, and TV

in clinician-collected and patient-collected vaginal swab specimens from symptomatic females. The Aptima CV/TV assay includes an internal control (IC).

Principles of the Procedure

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 IC in every test to monitor nucleic acid capture, amplification, and detection.

Specimens are collected in a tube containing Aptima® 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 that interprets the amplification signal emergence times to generate a Positive or Negative status for each target organism in the sample.

Summary of Safety and Performance

The SSP (Summary of Safety and Performance) is available in the European database on medical devices (Eudamed), where it is linked to the device identifiers (Basic UDI-DI). To locate the SSP for the Aptima CV/TV assay, refer to the Basic Unique Device Identifier (BUDI): **54200455DIAGAPTCVTV2E**.

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 refer to the *Panther/Panther Fusion® System Operator's Manual for procedural information* prior to performing the assay on the Panther system.

D. Only personnel adequately trained in the use of the Aptima CV/TV 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, precautions, and procedures to control contamination for the Panther system, consult 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 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.

I. Dispose of all materials that have come in contact with specimens and reagents in accordance with applicable national, international, and regional regulations. Thoroughly clean and disinfect all work surfaces.

J. Use good standard practices for molecular laboratories including environmental monitoring. See *Procedural Notes* for suggested Lab Contamination Monitoring Protocol for the Panther system.

Specimen Related

K. Expiration dates listed on the collection kits pertain to the collection site and not the testing facility. 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.

L. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established according to local regulations. Only personnel adequately trained in the use of the Aptima CV/TV assay and trained in handling infectious materials should perform this diagnostic procedure.

M. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers from different patients do not contact one another during specimen handling in the laboratory. Change gloves if they come in contact with a specimen.

N. Avoid cross-contamination by discarding used materials without passing over any other container.

O. 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.

P. 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.

Q. Upon piercing, liquid can discharge from Aptima transfer tube caps under certain conditions. Follow instructions in the *Panther System Test Procedure* to prevent this occurrence.

Assay Related

R. 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.

S. Use Universal Precautions when handling controls.

T. Avoid microbial and ribonuclease contamination of reagents.

U. Do not use the reagent, control, or calibrator kits after their expiration dates.

V. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Controls, the calibrator, and assay fluids may be interchanged.

W. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

X. Some reagents in this kit are labeled with hazard information.

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.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

EU Hazard Information	
—	**Amplification Reagent** *Magnesium Chloride 60 - 65%* — H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
—	**Enzyme Reagent** *HEPES 1 - 5%* *Triton X-100 1 - 5%* — H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.

—	**Enzyme Reconstitution Solution** *Glycerol 20 - 25%* *Triton X-100 5 - 10%* *HEPES 1 - 5%* — H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
—	**Promoter Reagent** *Magnesium Chloride 35 - 40%* — H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
—	**Target Capture Reagent** *HEPES 5 - 10%* *EDTA 1 - 5%* *Lithium Hydroxide, Monohydrate 1 - 5%* — H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.

Reagent Storage and Handling Requirements

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

		Open Kit (Reconstituted)	
Reagent	**Unopened Storage**	**Storage**	**Stability**
Amplification Reagent	2ºC to 8ºC	N/A	N/A
Amplification Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days[1]
Enzyme Reagent	2ºC to 8ºC	N/A	N/A
Enzyme Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days[1]
Promoter Reagent	2ºC to 8ºC	N/A	N/A
Promoter Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days[1]
Target Capture Reagent	15ºC to 30ºC	15ºC to 30ºC[2]	30 days[1]
Positive Calibrator	2ºC to 8ºC	N/A	Single Use Vial
Negative Control	2ºC to 8ºC	N/A	Single Use Vial
Positive Control	2ºC to 8ºC	N/A	Single Use Vial
Internal Control	2ºC to 8ºC	N/A	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. The 100-test assay kit can be loaded onto the Panther system up to 8 times. The 250-test assay kit can be loaded onto the Panther system up to 5 times. The system logs each time the reagents are loaded.

D. The 250-test assay kit Promoter Reagent bottle is the same size as the Enzyme Reagent bottle. After loading the Promoter Reagent bottle into the reagent rack, check that the bottle is fully pushed down.

E. Reagents stored on-board the Panther system have 120 hours of on-board stability.

F. Avoid cross-contamination during reagent handling and storage. Recap all reconstituted reagents with new reagent caps each time prior to storage.

G. The Promoter Reagent and reconstituted Promoter Reagent are photosensitive. Protect these reagents from light during storage and preparation for use.

H. 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 any other container.*

Vaginal swab specimens can be tested with the Aptima CV/TV 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 CV/TV assay.

1. Swab Specimens
   a. Option 1: After collection, swab specimens in transport tubes can be stored at 2ºC to 8ºC for up to 30 days. If longer storage is needed, specimens may be stored at -20ºC or -70ºC for an additional 60 days.
   b. Option 2: 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 assayed 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 frozen or 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 must 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 CV/TV assay are listed below for the Panther system. Reagent identification symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima CV/TV Assay Kit

100 tests: 2 assay boxes, 1 calibrator kit, and 1 controls kit (Cat. No. PRD-05189)

250 tests: 2 assay boxes, 1 calibrator kit, and 1 controls kit (Cat. No. PRD-07665)

Aptima CV/TV Assay Refrigerated Box (Box 1 of 2)
(store at 2ºC to 8ºC upon receipt)

Symbol	Component	Quantity	
		250-Test Kit	100-Test Kit
A	**Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial	1 vial
E	**Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial	1 vial
PRO	**Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial	1 vial
IC	**Internal Control** *Non-infectious nucleic acids in buffered solution.*	1 x 0.56 mL	1 x 0.3 mL

Aptima CV/TV Assay Room Temperature Box (Box 2 of 2)
(store at 15ºC to 30ºC upon receipt)

Symbol	Component	Quantity	
		250-Test Kit	100-Test Kit
AR	**Amplification Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 18.5 mL	1 x 7.2 mL
ER	**Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL	1 x 5.8 mL
PROR	**Promoter Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 11.9 mL	1 x 4.5 mL
TCR	**Target Capture Reagent** *Buffered salt solution containing non-infectious nucleic acids and magnetic particles.*	1 x 54.0 mL	1 x 26.0 mL
	Reconstitution Collars	3	3
	Master Lot Barcode Sheet	1 sheet	1 sheet

Aptima CV/TV Assay Calibrator Kit (PRD-05191)
(store at 2ºC to 8ºC upon receipt)

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

Aptima CV/TV Assay Controls Kit (PRD-05190)
(store at 2ºC to 8ºC upon receipt)

Symbol	Component	Quantity
CONTROL-	**Negative Control** *Buffered solution.*	5 x 2.7 mL
CONTROL+	**Positive Control** *Non-infectious C. albicans, C. glabrata,* and TV cultured organisms in buffered solution.	5 x 1.7 mL
	Control Barcode Label	1 sheet

Materials Required but Available Separately

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

Material	Cat. No.
Panther® System	303095
Panther Fusion® System	PRD-04172
Panther® System Continuous Fluids and Waste (Panther Plus)	PRD-06067
Aptima® CV/TV Assay Calibrator Kit	PRD-05191
Aptima® CV/TV Assay Controls Kit	PRD-05190
Panther Run Kit for Real Time Assays (for real time assays only)	PRD-03455 (5000 tests)
Aptima® Assay Fluids Kit (also known as Universal Fluids Kit) *Contains Aptima® Wash Solution, Aptima® Buffer for Deactivation Fluid, and Aptima® Oil Reagent*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther® Waste Bag Kit	902731
Panther® Waste Bin Cover	504405
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*	303096 (5000 tests)

Material	Cat. No.
Aptima Assay Fluids Kit *Contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable. *Not all products are available in all regions. Contact your representative for region-specific information*	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 for the 100-test kits *Amplification, Enzyme, and Promoter reagent reconstitution bottles* *TCR bottle*	 CL0041 (100 caps) 501604 (100 caps)
Reagent Replacement Caps for the 250-test kits *Amplification reagent reconstitution bottle* *Enzyme and Promoter reagent reconstitution bottles* *TCR bottle*	 CL0041 (100 caps) 501616 (100 caps) CL0040 (100 caps)
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—

Optional Materials

Material	Cat. No.
Hologic® Bleach Enhancer for Cleaning *For routine cleaning of surfaces and equipment*	302101
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. Cover

the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

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. Label caps of reconstitution solution bottles.
   d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass 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. Pick up the assembled bottles and swirl the assembled bottles for at least 10 seconds. Avoid creating foam while swirling the bottle (Figure 1, Step 4).
   i. Wait at least 15 minutes to ensure the lyophilized reagent goes completely into solution. Swirl the bottles again for at least 10 seconds and then slightly rock the solution within the glass vial back and forth to mix thoroughly.
   j. Visually check to see if reagent is completely in solution with no powder, clumps, or wavy lines.
   k. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle (Figure 1, Step 5).
   l. Remove the reconstitution collar and glass vial (Figure 1, Step 6).
   m. Recap the plastic bottle with either the saved, labeled cap that corresponds to the reagent or a new cap. Do not mismatch caps. Record operator initials and reconstitution date on the label (Figure 1, Step 7).
   n. Discard the reconstitution collar and glass vial (Figure 1, Step 8).
   o. Thoroughly mix each reagent by gently inverting prior to loading onto the Panther system.

Option: Additional mixing of the Amplification, Enzyme, and Promoter reagents is allowed by placing the recapped plastic bottles on a tube rocker set at a moderate speed and tilt for a minimum of 5 minutes. Ensure reagents are thoroughly mixed.

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

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

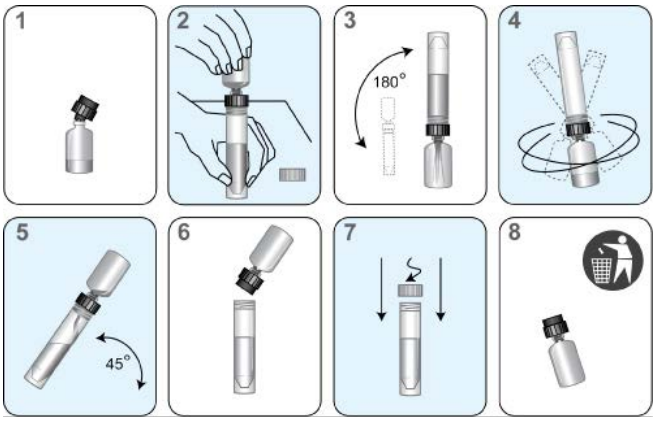

Figure 1. Reagent Reconstitution Process

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 reconstituted Amplification, Enzyme, and Promoter reagents capped plastic bottles may be placed on a tube rocker set at a moderate speed and tilt for a minimum of 25 minutes to ensure reagents reach room temperature and are thoroughly mixed.

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 on-board 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.

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

D. Calibrator and Control Preparation

1. Remove the calibrator and controls from storage (2°C to 8°C) and allow the calibrator and controls to reach room temperature (15°C to 30°C) prior to processing.

E. Specimen Handling

1. 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.
2. Allow the specimens to reach room temperature (15°C to 30°C) prior to processing.

   Note: *Prior to testing and/or to resolve suspected specimen related invalid results, specimen may be vortexed at high speed for a minimum of 3 minutes, followed by low speed vortexing for 1 minute (to draw the fluid down into the tube).*
3. 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 3a-3b 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.*

F. 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.
2. Load samples.

Procedural Notes

A. Calibrator and Controls

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. Glove Powder

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

C. Lab Contamination Monitoring Protocol for the Panther System

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence, and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the Aptima Multitest Swab Specimen Collection Kit:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab from its packaging, wet the swab in the swab transport medium, and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.
7. Test samples with the Aptima CV/TV assay on the Panther system.
8. Further investigation should be performed if any samples yield a positive result.

For test interpretation, see *Test Interpretation*. For additional Panther system-specific contamination monitoring information, contact Hologic Technical Support.

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 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. Detection of the internal control is not required for samples that are C spp, *C. glabrata*, and/or TV positive.

The IC must be detected in all samples that are negative for C spp, *C. glabrata*, and/or TV; samples that fail to meet that criteria will be reported as invalid. Each sample with an invalid result must be retested.

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. Results for C spp, *C. glabrata*, and TV detection are reported separately. The table below shows the possible results reported in a valid run and result interpretations. The first valid result for each analyte is the result that should be reported. Samples with invalid test results should be retested. If the result is invalid upon retest, a new specimen should be collected.

Table 1: Result Interpretation

C spp Result[1]	*C. glabrata* Result	TV Result	Result[2]	Interpretation
Positive	Negative	Negative	Valid	C spp RNA detected.
Positive	Positive	Negative	Valid	C spp RNA and *C. glabrata* RNA detected.
Positive	Negative	Positive	Valid	C spp RNA and TV RNA detected.
Positive	Positive	Positive	Valid	C spp RNA, *C. glabrata* RNA, and TV RNA detected.
Negative	Positive	Negative	Valid	*C. glabrata* RNA detected.
Negative	Negative	Positive	Valid	TV RNA detected.
Negative	Positive	Positive	Valid	*C. glabrata* RNA and TV RNA detected.
Negative	Negative	Negative	Valid	Negative for C spp, *C. glabrata*, and TV.
Invalid	Invalid	Invalid	Invalid	Invalid: there was an error in the generation of the result. Specimen should be retested.

[1]C spp species group RNA = *C. albicans, C. parapsilosis, C. dubliniensis,* and/or *C. tropicalis.*

[2]The valid or invalid status of the reaction is shown in the Result column. The Result column considers the internal control and positive or negative status of analytes.

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, proper specimen collection techniques are necessary. See *Specimen Collection and Storage* for instructions. Refer to the package insert of the appropriate Hologic specimen collection kit.

E. Therapeutic failure or success cannot be determined with the Aptima CV/TV assay since nucleic acid may persist following appropriate antimicrobial therapy.

F. Results from the Aptima CV/TV assay should be interpreted in conjunction with other clinical data available to the clinician.

G. A negative result does not preclude a possible infection because results are dependent on adequate specimen collection. Test results may be affected by improper specimen collection, technical error, specimen mix-up, or target levels below the assay limit of detection (LoD).

H. The Aptima CV/TV 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. A *Candida* species group positive result can be due to one or multiple *Candida* species.

J. The performance of the Aptima CV/TV assay has not been evaluated in adolescents less than 14 years of age.

K. Customers must independently validate an LIS transfer process.

L. The Aptima CV/TV assay has not been evaluated for use with specimens collected by patients at home.

M. Collection and testing of patient-collected vaginal swab specimens with the Aptima CV/TV assay is not intended to replace clinical examination. Vaginal infections may result from other causes or concurrent infections may occur.

N. Interference with the Aptima CV/TV assay was observed in the presence of the following substances: tioconazole 6.5% ointment (3% W/V, all analytes), vaginal moisturizing gel (1% W/V, C spp; 5% W/V, *C. glabrata*; 3% W/V, TV), and glacial acetic acid (5% V/V, C spp only).

O. The following organism was observed to cross-react above the stated concentrations: *Candida famata* at concentrations higher than $5x10^5$ CFU/mL.

P. Competitive interference was observed in co-infected samples for the combination of low *C. glabrata* (3X LoD) and high TV ($1x10^5$ or $1x10^4$ cells/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 *Candida* and TV in patient populations depends on age, ethnicity, risk factors, the type of clinic, and the sensitivity of the test used to detect infections. A summary of the positivity of C spp, *C. glabrata*, and TV detection in symptomatic subjects, as determined by the Aptima CV/TV assay on the Panther system, is shown in Table 2 for the multicenter study, by clinical site and overall.

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

	% Positivity (# positive/# tested with valid results)					
	Clinician-collected Vaginal Swabs			Patient-collected Vaginal Swabs		
Site	C spp group[1]	*C. glabrata*	TV	C spp group[1]	*C. glabrata*	TV
1	15.0 (3/20)	5.0 (1/20)	6.3 (1/16)	20.0 (4/20)	5.0 (1/20)	6.3 (1/16)
2	20.0 (1/5)	0.0 (0/5)	0.0 (0/1)	0.0 (0/5)	0.0 (0/5)	0.0 (0/1)
3	54.5 (12/22)	0.0 (0/22)	9.5 (2/21)	54.5 (12/22)	0.0 (0/22)	9.5 (2/21)
4	23.1 (50/216)	5.1 (11/216)	30.5 (65/213)	28.2 (60/213)	7.0 (15/213)	18.0 (38/211)
5	25.9 (38/147)	4.8 (7/146)	9.0 (13/145)	28.5 (41/144)	5.6 (8/144)	7.7 (11/143)
6	33.3 (24/72)	4.2 (3/72)	2.9 (2/68)	33.3 (24/72)	4.2 (3/72)	1.5 (1/68)
7	24.4 (48/197)	7.6 (15/197)	36.5 (72/197)	27.9 (55/197)	7.1 (14/197)	28.9 (57/197)
8	0.0 (0/1)	0.0 (0/1)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)	100.0 (1/1)
9	38.0 (41/108)	1.9 (2/108)	3.8 (4/105)	46.3 (50/108)	2.8 (3/108)	3.8 (4/105)
10	47.1 (8/17)	5.9 (1/17)	0.0 (0/17)	52.9 (9/17)	5.9 (1/17)	0.0 (0/17)
11	26.8 (19/71)	5.6 (4/71)	11.4 (8/70)	27.8 (20/72)	5.6 (4/72)	5.6 (4/71)
12	33.3 (46/138)	2.9 (4/138)	2.3 (3/130)	34.1 (46/135)	3.0 (4/135)	2.3 (3/129)
13	30.4 (21/69)	1.4 (1/69)	13.0 (9/69)	31.9 (22/69)	2.9 (2/68)	11.6 (8/69)
14	44.4 (4/9)	0.0 (0/9)	0.0 (0/8)	44.4 (4/9)	0.0 (0/9)	0.0 (0/8)
15	50.0 (2/4)	0.0 (0/4)	0.0 (0/4)	50.0 (2/4)	0.0 (0/4)	0.0 (0/4)
16	40.0 (12/30)	3.3 (1/30)	10.7 (3/28)	46.7 (14/30)	3.3 (1/30)	10.7 (3/28)
17	37.5 (30/80)	2.5 (2/80)	2.7 (2/74)	40.0 (32/80)	1.3 (1/80)	4.1 (3/74)
18	36.0 (31/86)	1.2 (1/85)	4.8 (4/83)	37.2 (32/86)	1.2 (1/85)	4.8 (4/83)
19	44.0 (33/75)	5.3 (4/75)	2.8 (2/71)	48.0 (36/75)	5.3 (4/75)	2.8 (2/71)
20	10.3 (4/39)	5.1 (2/39)	0.0 (0/39)	10.3 (4/39)	5.1 (2/39)	0.0 (0/39)
21	20.3 (16/79)	5.1 (4/79)	11.5 (9/78)	25.3 (20/79)	5.1 (4/79)	10.4 (8/77)

Table 2: Positivity as Determined by the Aptima CV/TV Assay in Symptomatic Women by Specimen Type and Clinical Site (continued)

	% Positivity (# positive/# tested with valid results)					
	Clinician-collected Vaginal Swabs			Patient-collected Vaginal Swabs		
Site	**C spp group[1]**	***C. glabrata***	**TV**	**C spp group[1]**	***C. glabrata***	**TV**
All	29.8 (443/1485)	4.2 (63/1483	13.9 (200/1438)	33.0 (487/1477)	4.6 (68/1475)	10.5 (150/1433)

[1] *C* spp species group RNA=*C. albicans, C. parapsilosis, C. dubliniensis,* and/or *C. tropicalis.*

Panther System Assay Performance

Reproducibility

Aptima CV/TV 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 STM spiked with simulated vaginal fluid negative for *Candida* species and TV. Six positive panel members were created by spiking the SVSM matrix with approximately 2X C_{95} or LoD (low-positive) or 3X C_{95} or LoD (moderate positive) concentrations of whole cell lysates positive for *C. albicans*, *C. glabrata*, or TV. 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 CV/TV 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 runs, and overall, is shown in Table 3.

Table 3: Signal Variability by Positive Panel Members

Panel Description	N	Mean TTime[1]	Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
C. albicans Low Pos[1]	108	14.68	0.66	4.47	0.00	0.00	0.00	0.00	0.41	2.78	0.30	2.02	0.83	5.64
C. albicans Mod Pos[1]	107	14.37	0.66	4.58	0.14	0.99	0.00	0.00	0.35	2.42	0.28	1.98	0.81	5.64
C. glabrata Low Pos	106	21.36	0.84	3.94	0.18	0.84	0.00	0.00	0.68	3.17	0.62	2.89	1.26	5.88
C. glabrata Mod Pos	107	20.54	0.99	4.83	0.30	1.46	0.00	0.00	0.76	3.70	0.48	2.34	1.37	6.68
TV Low Pos	108	24.32	1.16	4.77	0.00	0.00	0.00	0.00	0.90	3.71	0.60	2.48	1.59	6.54
TV Mod Pos	107	23.09	1.18	5.13	0.00	0.00	0.00	0.00	0.86	3.71	0.56	2.41	1.56	6.77

CV = coefficient of variation, Mod = moderate, Pos = positive, SD = standard deviation, TTime = threshold time.

[1] C_{95} (*C. albicans* panels) is defined relative to clinical cutoff.

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, multicenter clinical study was conducted to establish the clinical performance characteristics of the Aptima CV/TV 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, sexually transmitted infections (STI), and medical group clinics, and clinical research centers.

Five (5) 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 CV/TV assay testing, and three additional vaginal swab samples were collected for reference testing. The following reference methods were used for all subjects:

- C spp and *C. glabrata* infection statuses were determined separately using Sabouraud dextrose and chromogenic culture of a clinician-collected swab sample, followed by PCR/bi-directional sequencing. For subjects with positive culture results (i.e., growth of any *Candida* 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. A positive sequencing result for C spp in either Aptima swab sample type was sufficient to establish a reference result positive for C spp in both Aptima swab types, and either a negative *Candida* culture result or a negative PCR/bi-directional sequencing result for both Aptima swab samples was sufficient to establish a reference result negative for C spp in both Aptima swab types; a similar algorithm was followed for establishing *C. glabrata* reference results.
- TV patient infection status (PIS) was determined using a composite result from two FDA-cleared assays for TV, one molecular assay and one culture-based assay. A positive result for at least one assay was sufficient to establish a reference result positive for TV for both Aptima swab types, and a negative result for both assays was sufficient to establish a reference result negative for TV for both Aptima swab types.

Aptima samples were tested with the Aptima CV/TV assay on the Panther system at three sites.

Performance characteristics for each prospectively-collected sample type, with corresponding 2-sided 95% Score confidence intervals (CIs), were estimated relative to C Spp and *C. glabrata* infection status and TV PIS.

Of the 1519 symptomatic subjects enrolled, 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 *Candida* infection status or TV PIS (n = 4). The remaining 1496 subjects were evaluable for at least one analyte in at least one of the sample types. Table 4 shows the demographics of evaluable subjects.

Table 4: Demographics of Evaluable Subjects

Characteristics		Total
Total, N	N	1496
Age (years)	Mean ± SD	35.3 ± 11.76
	Median	33.0
	Range	14-79
Age category (years), n (%)	14-17	5 (0.3)
	18-29	554 (37.0)
	30-39	480 (32.1)
	40-49	247 (16.5)
	>50	210 (14.0)
Ethnicity, n (%)	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 ethnicities.

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 TV.

Candida Species Group Performance Characteristics

The sensitivity and specificity of the Aptima CV/TV assay for the detection of C spp are shown for both sample types overall and by site in Table 5. Assay performance is shown stratified by ethnicity in Table 6, and by clinical condition in Table 7.

Table 5: Candida Species Group Performance Characteristics by Collection Site in Symptomatic Women

	Clinician-collected Vaginal Swabs				Patient-collected Vaginal Swabs			
Site	**N**	**Prev (%)**	**Sensitivity % (95% CI)[1]**	**Specificity % (95% CI)[1]**	**N**	**Prev (%)**	**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**
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

Table 5: Candida Species Group Performance Characteristics by Collection Site in Symptomatic Women (continued)

	Clinician-collected Vaginal Swabs				Patient-collected Vaginal Swabs			
Site	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
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 (20.7-100) 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

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

[1] Score CI.

Table 6: Candida Species Group Performance Characteristics by Ethnicity in Symptomatic Women

Specimen Type	Ethnicity	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
Clinician-collected Vaginal Swabs	**All**	**1485**	**28.6**	**91.7 (88.7-94.0) 389/424**	**94.9 (93.4-96.1) 1007/1061**
	Asian	73	26.0	100 (83.2-100) 19/19	94.4 (84.9-98.1) 51/54
	Black/African-American	747	30.4	90.7 (86.3-93.9) 206/227	94.0 (91.7-95.8) 489/520
	White (Hispanic/Latino)	265	28.7	93.4 (85.5-97.2) 71/76	93.7 (89.2-96.3) 177/189
	White (Not Hispanic/Latino)	336	23.8	91.3 (83.0-95.7) 73/80	97.7 (95.0-98.9) 250/256
	Other[2]	64	34.4	90.9 (72.2-97.5) 20/22	95.2 (84.2-98.7) 40/42
Patient-collected Vaginal Swabs	**All**	**1477**	**28.6**	**92.9 (90.0-95.0) 392/422**	**91.0 (89.1-92.6) 960/1055**
	Asian	71	25.4	100 (82.4-100) 18/18	90.6 (79.7-95.9) 48/53
	Black/African-American	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	89.9 (84.8-93.5) 170/189
	White (Not Hispanic/Latino)	332	23.5	96.2 (89.3-98.7) 75/78	95.3 (91.9-97.3) 242/254
	Other[2]	64	34.4	95.5 (78.2-99.2) 21/22	90.5 (77.9-96.2) 38/42

CI = confidence interval, Prev = prevalence.

[1] Score CI.

[2] Includes patient-reported other, mixed, and unknown ethnicities.

Table 7: Candida Species Group 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**	**1485**	**28.6**	**91.7 (88.7-94.0) 389/424**	**94.9 (93.4-96.1) 1007/1061**
	Use of antibiotics	5	60.0	66.7 (20.8-93.9) 2/3	50.0 (9.5-90.5) 1/2
	Use of antifungals	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
	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
	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
	Pregnant	20	55.0	100 (74.1-100) 11/11	100 (70.1-100) 9/9
	With menses	118	30.5	94.4 (81.9-98.5) 34/36	97.6 (91.5-99.3) 80/82
	Without menses	1210	29.6	92.5 (89.2-94.8) 331/358	94.4 (92.6-95.7) 804/852
	Post-menopausal	157	19.1	80.0 (62.7-90.5) 24/30	96.9 (92.2-98.8) 123/127
Patient-collected Vaginal Swabs	**All**	**1477**	**28.6**	**92.9 (90.0-95.0) 392/422**	**91.0 (89.1-92.6) 960/1055**
	Use of antibiotics	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
	Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2
	Recurrent symptoms of vaginitis in the last 12 months	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	95	27.4	88.5 (71.0-96.0) 23/26	85.5 (75.3-91.9) 59/69
	Pregnant	21	52.4	100 (74.1-100) 11/11	100 (72.2-100) 10/10
	With menses	116	30.2	97.1 (85.5-99.5) 34/35	88.9 (80.2-94.0) 72/81
	Without menses	1207	29.7	93.0 (89.9-95.2) 333/358	91.0 (88.9-92.8) 773/849
	Post-menopausal	154	18.8	86.2 (69.4-94.5) 25/29	92.0 (85.9-95.6) 115/125

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.

Candida glabrata Performance Characteristics

The sensitivity and specificity of the Aptima CV/TV assay for the detection of *C. glabrata* are shown for both sample types overall and by site in Table 8. Assay performance is shown stratified by ethnicity in Table 9, and by clinical condition in Table 10.

Table 8: Candida glabrata Performance Characteristics by Collection Site in Symptomatic Women

	Clinician-collected Vaginal Swabs				Patient-collected Vaginal Swabs			
Site	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
All	**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	216	5.6	66.7 (39.1-86.2) 8/12	98.5 (95.8-99.5) 200/203	213	5.6	75.0 (46.8-91.1) 9/12	97.0 (93.6-98.6) 195/201
5	146	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 (20.7-100) 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

Table 8: Candida glabrata Performance Characteristics by Collection Site in Symptomatic Women (continued)

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

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

[1] Score CI.

[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.

Table 9: Candida glabrata Performance Characteristics by Ethnicity in Symptomatic Women

Specimen Type	Ethnicity	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
Clinician-collected Vaginal Swabs	**All**	**1483**	**4.0**	**84.7 (73.5-91.8) 50/59**	**99.1 (98.4-99.5) 1411/1424**
	Asian	72	4.2	100 (43.9-100) 3/3	100 (94.7-100) 69/69
	Black/African-American	747	4.1	74.2 (56.8-86.3) 23/31	98.7 (97.6-99.3) 707/716
	White (Hispanic/Latino)	264	3.0	87.5 (52.9-97.8) 7/8	99.6 (97.8-99.9) 255/256
	White (Not Hispanic/Latino)	336	4.2	100 (78.5-100) 14/14	99.1 (97.3-99.7) 319/322
	Other[2]	64	4.7	100 (43.9-100) 3/3	100 (94.1-100) 61/61

Table 9: Candida glabrata Performance Characteristics by Ethnicity in Symptomatic Women (continued)

Specimen Type	Ethnicity	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
Patient-collected Vaginal Swabs	**All**	**1475**	**3.9**	**86.2 (75.1-92.8) 50/58**	**98.7 (98.0-99.2) 1399/1417**
	Asian	71	4.2	100 (43.9-100) 3/3	98.5 (92.1-99.7) 67/68
	Black/African-American	744	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	99.2 (97.2-99.8) 254/256
	White (Not Hispanic/Latino)	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	98.4 (91.3-99.7) 60/61

CI = confidence interval, Prev = prevalence.

[1] Score CI.

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

Table 10: Candida glabrata 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**	**1483**	**4.0**	**84.7 (73.5-91.8) 50/59**	**99.1 (98.4-99.5) 1411/1424**
	Use of antibiotics	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
	Use of estrogen therapy	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
	Unprotected intercourse in the last 24 hours	96	4.2	100 (51.0-100) 4/4	100 (96.0-100) 92/92
	Pregnant	20	0.0	NC	95.0 (76.4-99.1) 19/20
	With menses	117	2.6	100 (43.9-100) 3/3	100 (96.7-100) 114/114
	Without menses	1209	3.8	80.4 (66.8-89.3) 37/46	99.1 (98.4-99.5) 1153/1163
	Post-menopausal	157	6.4	100 (72.2-100) 10/10	98.0 (94.2-99.3) 144/147

Table 10: Candida glabrata Performance Characteristics by Clinical Condition in Symptomatic Women (continued)

Collection Type	Clinical Condition	N[1]	Prev (%)	Sensitivity % (95% CI)[2]	Specificity % (95% CI)[2]
Patient-collected Vaginal Swabs	**All**	**1475**	**3.9**	**86.2 (75.1-92.8) 50/58**	**98.7 (98.0-99.2) 1399/1417**
	Use of antibiotics	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
	Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2
	Recurrent symptoms of vaginitis in the last 12 months	858	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	95	4.2	100 (51.0-100) 4/4	100 (95.9-100) 91/91
	Pregnant	21	0.0	NC	90.5 (71.1-97.3) 19/21
	With menses	116	2.6	100 (43.9-100) 3/3	100 (96.7-100) 113/113
	Without menses	1205	3.8	84.8 (71.8-92.4) 39/46	99.0 (98.2-99.4) 1147/1159
	Post-menopausal	154	5.8	88.9 (56.5-98.0) 8/9	95.9 (91.3-98.1) 139/145

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.

Due to anticipated low prevalence of *C. glabrata*, the performance of the Aptima CV/TV assay was also assessed using contrived specimens to supplement the data collected in the clinical study. Contrived specimens were prepared by spiking five different strains of *C. glabrata* in simulated vaginal swab matrix, at concentrations of 3X, 10X, and 20X the assay's LoD. True negative specimens containing matrix only were also tested. Agreement was 100% across all contrived specimens (see Table 11).

Table 11: Candida glabrata Contrived Specimen Agreement

	N	*C. glabrata* Positive	*C. glabrata* Negative	PPA % (95% CI)[1]	NPA % (95% CI)[1]
True Negative	60	0	60	NC	100 (94.0-100)
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

NC = not calculable, LoD = limit of detection, NPA = negative percent agreement, PPA = positive percent agreement.

[1] Score CI.

Trichomonas vaginalis Performance Characteristics

The sensitivity and specificity of the Aptima CV/TV assay for the detection of TV are shown for both sample types overall and by site in Table 12. Assay performance is shown stratified by ethnicity in Table 13, and by clinical condition in Table 14.

Table 12: Trichomonas vaginalis Performance Characteristics by Collection Site in Symptomatic Women

	Clinician-collected Vaginal Swabs				Patient-collected Vaginal Swabs			
Site	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
All	**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 (20.7-100) 1/1	NC	1	100.0	100 (20.7-100) 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 (81.6-100) 17/17
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 (94.5-100) 66/66
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

Table 12: Trichomonas vaginalis Performance Characteristics by Collection Site in Symptomatic Women (continued)

	Clinician-collected Vaginal Swabs				Patient-collected Vaginal Swabs			
Site	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
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) 25/25
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) 71/72
18	83	4.8	100 (51.0-100) 4/4	100 (95.4-100) 79/79	83	4.8	100 (51.0-100) 4/4	100 (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

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

[1] Score CI.

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

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

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

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

Table 13: Trichomonas vaginalis Performance Characteristics by Ethnicity in Symptomatic Women

Specimen Type	Ethnicity	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
Clinician-collected Vaginal Swabs	**All**	**1438**	**9.9**	**96.5 (92.0-98.5) 137/142**	**95.1 (93.8-96.2) 1233/1296**
	Asian	67	6.0	100 (51.0-100) 4/4	98.4 (91.5-99.7) 62/63
	Black/African-American	727	14.2	98.1 (93.2-99.5) 101/103	93.3 (91.0-95.0) 582/624
	White (Hispanic/Latino)	257	6.6	94.1 (73.0-99.0) 16/17	95.0 (91.5-97.1) 228/240
	White (Not Hispanic/Latino)	326	4.0	84.6 (57.8-95.7) 11/13	97.4 (95.0-98.7) 305/313
	Other[2]	61	8.2	100 (56.6-100) 5/5	100 (93.6-100) 56/56

Table 13: Trichomonas vaginalis Performance Characteristics by Ethnicity in Symptomatic Women (continued)

Specimen Type	Ethnicity	N	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]
Patient-collected Vaginal Swabs	**All**	**1433**	**9.8**	**97.1 (92.9-98.9) 136/140**	**98.9 (98.2-99.4) 1279/1293**
	Asian	66	6.1	100 (51.0-100) 4/4	100 (94.2-100) 62/62
	Black/African-American	724	14.0	98.0 (93.1-99.5) 99/101	98.7 (97.5-99.3) 615/623
	White (Hispanic/Latino)	258	6.6	94.1 (73.0-99.0) 16/17	97.9 (95.2-99.1) 236/241
	White (Not Hispanic/Latino)	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

CI = confidence interval, Prev = prevalence.

[1] Score CI.

[2] Includes patient-reported other, mixed, and unknown ethnicities.

Table 14: Trichomonas vaginalis 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**	**1438**	**9.9**	**96.5 (92.0-98.5) 137/142**	**95.1 (93.8-96.2) 1233/1296**
	Use of antibiotics	5	0.0	NC	100 (56.6-100) 5/5
	Use of antifungals	7	0.0	NC	100 (64.6-100) 7/7
	Use of estrogen therapy	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
	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
	Pregnant	20	15.0	66.7 (20.8-93.9) 2/3	100 (81.6-100) 17/17
	With menses	112	9.8	90.9 (62.3-98.4) 10/11	97.0 (91.6-99.0) 98/101
	Without menses	1176	9.9	97.4 (92.7-99.1) 114/117	95.3 (93.8-96.4) 1009/1059
	Post-menopausal	150	9.3	92.9 (68.5-98.7) 13/14	92.6 (87.0-96.0) 126/136

Table 14: Trichomonas vaginalis Performance Characteristics by Clinical Condition in Symptomatic Women

Collection Type	Clinical Condition	N[1]	Prev (%)	Sensitivity % (95% CI)[2]	Specificity % (95% CI)[2]
Patient-collected Vaginal Swabs	**All**	**1433**	**9.8**	**97.1 (92.9-98.9) 136/140**	**98.9 (98.2-99.4) 1279/1293**
	Use of antibiotics	5	0.0	NC	100 (56.6-100) 5/5
	Use of antifungals	7	0.0	NC	100 (64.6-100) 7/7
	Use of estrogen therapy	2	0.0	NC	100 (34.2-100) 2/2
	Recurrent symptoms of vaginitis in the last 12 months	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	93	12.9	100 (75.8-100) 12/12	100 (95.5-100) 81/81
	Pregnant	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	99.0 (94.6-99.8) 100/101
	Without menses	1173	9.8	97.4 (92.6-99.1) 112/115	98.9 (98.0-99.4) 1046/1058
	Post-menopausal	148	9.5	100 (78.5-100) 14/14	99.3 (95.9-99.9) 133/134

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.

Co-detection rates, calculated for specimens with valid and conclusive Aptima CV/TV assay and reference results for all targets are reported in Table 15.

Table 15: Aptima CV/TV Assay Co-detection Rates in Symptomatic Women

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

Positivity Rates in Asymptomatic Women

The detection of an imbalance in the vaginal microbiome is relevant for treatment decisions. Although the Aptima CV/TV assay is not intended for use in testing samples from asymptomatic women, organisms associated with vulvovaginal candidiasis and detected by the Aptima CV/TV assay may also be present in asymptomatic women. Presence of the Aptima CV/TV assay targets was assessed in clinician-collected vaginal swab samples from 171 asymptomatic women. A summary of the detection rates for C spp and *C. glabrata* as determined by the Aptima CV/TV assay, is shown in Table 16 for the multicenter study overall and by ethnicity.

Table 16: Positivity as Determined by the Aptima CV/TV Assay in Asymptomatic Women

	% Positivity (# positive/# tested with valid results)	
	C spp group	***C. glabrata***
All	**21.1% (36/171)**	**8.8% (15/171)**
Asian	0.0% (0/5)	0.0% (0/5)
Black/African American	28.0% (21/75)	12.0% (9/75)
White (Hispanic/Latino)	17.1% (7/41)	4.9% (2/41)
White (Not Hispanic/Latino)	11.6% (5/43)	7.0% (3/43)
Other[1]	42.9% (3/7)	14.3% (1/7)

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

Invalid Rates

A total of 3295 clinician- and patient-collected samples from symptomatic and asymptomatic subjects were processed in valid Aptima CV/TV assay runs to establish clinical performance. Of these, 1.7% had initial invalid results. Upon retest, 0.5% remained invalid and were excluded from all analyses.

Panther System Analytical Performance

Analytical Sensitivity

The analytical sensitivity/LoD of the Aptima CV/TV assay was determined by testing a series of panels consisting of target organisms diluted in pooled negative clinical specimens or SVSM. A minimum of 20 replicates of each panel member were tested with each of the two reagent lots, for a minimum of 40 replicates per panel member. Probit analysis was performed to generate the 95% predicted detection limit for each organism. The predicted detection limits are shown in Table 17.

Table 17: Limit of Detection of the Aptima CV/TV Assay

Organism	Predicted Detection Limit	Concentration	Units
C. albicans	95%	4439	CFU/mL
C. glabrata	95%	41	CFU/mL
C. parapsilosis[1]	95%	9416	CFU/mL
C. tropicalis[1]	95%	811	CFU/mL
C. dubliniensis[1]	95%	1176	CFU/mL
TV	95%	0.0024	Cells/mL

CFU = colony forming units.

[1] Tested in simulated vaginal swab matrix.

Analytical Inclusivity

Five strains of each *Candida* target organism were tested using lysate targeting 3X LoD for *C. albicans*, *C. parapsilosis, C. tropicalis, C. dubliniensis* and *C. glabrata* in SVSM. Nine strains of TV 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. Eight of the nine TV strains, including the metronidazole resistant strain, were detected at 3X LoD. One strain of TV was detected at 4X LoD.

Cross-Reactivity and Microbial Interference

Cross-reactivity and microbial interference with the Aptima CV/TV assay were evaluated in the presence of closely related and non-targeted organisms. A panel consisting of 64 organisms and human cell lines (Table 18) was tested in SVSM in the absence or presence of 3X LoD *C. albicans*, *C. glabrata* or TV. No cross-reactivity or microbial interference was observed for any of the 64 organisms tested in the Aptima CV/TV assay at the concentrations listed in Table 18.

Table 18: Cross-Reactivity and Microbial Interference Panel

Microorganism	Concentration	Microorganism	Concentration
Acinetobacter lwoffii	$1x10^6$ CFU/mL	Herpes simplex virus I	$1x10^4$ TCID 50/mL
Actinomyces israelii	$1x10^6$ CFU/mL	Herpes simplex virus II	$1x10^4$ TCID 50/mL
Alcaligenes faecalis	$1x10^6$ CFU/mL	*Klebsiella pneumoniae*	$1x10^6$ CFU/mL
Atopobium vaginae	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^6$ CFU/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Lactobacillus crispatus*	$1x10^6$ CFU/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Lactobacillus gasseri*	$1x10^6$ CFU/mL
BVAB-1[1]	$1x10^6$ copies/mL	*Lactobacillus iners*	$1x10^6$ CFU/mL

Table 18: Cross-Reactivity and Microbial Interference Panel (continued)

Microorganism	Concentration	Microorganism	Concentration
BVAB-2[1]	$1x10^6$ copies/mL	*Lactobacillus jensenii*	$1x10^6$ CFU/mL
Campylobacter jejuni	$1x10^6$ CFU/mL	*Lactobacillus mucosae*	$1x10^6$ CFU/mL
Candida catenulata	$1x10^6$ CFU/mL	*Leptotrichia buccalis*	$1x10^6$ CFU/mL
Candida famata[2]	$5x10^5$ CFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Candida guilliermondii	$1x10^6$ CFU/mL	*Megasphaera Type 1*[1]	$1x10^6$ copies/mL
Candida haemulonii	$1x10^6$ CFU/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Candida inconspicua	$1x10^6$ CFU/mL	*Mycoplasma genitalium*	$1x10^6$ CFU/mL
Candida kefyr	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^6$ CFU/mL
Candida krusei	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Candida lusitaniae	$1x10^6$ CFU/mL	*Peptostreptococcus magnus*	$1x10^6$ CFU/mL
Candida norvegica	$1x10^6$ CFU/mL	*Pentatrichomonas hominis*	$1x10^5$ cells/mL
Candida orthopsilosis	$1x10^6$ CFU/mL	*Pichia fermentans*	$1x10^6$ CFU/mL
Chlamydia trachomatis	$1x10^6$ IFU/mL	*Prevotella bivia*	$1x10^6$ CFU/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Propionibacterium acnes*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	SiHa cells	$1x10^4$ cells/mL
Eggerthella lenta	$1x10^6$ CFU/mL	*Sneathia amnii*	$1x10^6$ CFU/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Escherichia coli	$1x10^6$ CFU/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
Fusobacterium nucleatum	$1x10^6$ CFU/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
Gardnerella vaginalis	$1x10^6$ CFU/mL	*Treponema pallidum*[1]	$1x10^6$ copies/mL
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Trichomonas tenax*	$1x10^5$ cells/mL
HeLa cells	$1x10^4$ Cells/mL	*Ureaplasma parvum*	$1x10^6$ CFU/mL
HIV	$1x10^5$ copies/mL	*Ureaplasma urealyticum*	$1x10^6$ CFU/mL

CFU = colony forming units; IFU = inclusion forming units; TCID50 = median tissue culture infectious dose.

[1] *In vitro* transcript tested.

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

Interference

Potentially interfering substances were tested in the Aptima CV/TV assay. Panels were built in SVSM and evaluated for potential effects on assay sensitivity and specificity. Sensitivity performance was evaluated separately for *C. albicans, C. glabrata,* and TV by spiking lysate at 3X LoD. 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 19.

Table 19: Interfering Substances Panel

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

Table 19: Interfering Substances Panel (continued)

Substance	Final Concentration[1]
Tioconazole[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 ≥1% W/V for *C. albicans*, 5% W/V for *C. glabrata,* and ≥3% W/V for TV. No interference was observed at 0.5% W/V for *C. albicans*, 4% W/V for *C. glabrata,* and 2% W/V for TV.

[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 TV.

Within Laboratory Precision

Within Lab Precision was evaluated on three Panther systems at one site. Three operators performed testing across 22 days and three reagent lots. Each operator performed two runs per day using a seven member panel. Each run consisted of three replicates of each panel member.

The panel members were made with *C. albicans*, *C. glabrata* or TV in SVSM. The six positive panel members targeted *C. albicans* at Low and Moderate Positive, *C. glabrata* at Low and Moderate Positive, and TV at Low and Moderate Positive. One Negative panel member contained matrix with no added target analytes.

The CV/TV percent positive results are presented in Table 20. Signal variability (TTime) of the Aptima CV/TV assay 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 21.

Table 20: Precision - Agreement of Aptima CV/TV Assay with Expected Results

Panel (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 (TV)	162/162	≥95%	100 (97.7-100.0)
Moderate Positive (*C. albicans*)	162/162	≥95%	100 (97.7-100.0)
Moderate Positive (*C. glabrata*)	162/162	≥95%	100 (97.7-100.0)
Moderate Positive (TV)	162/162	≥95%	100 (97.7-100.0)

Table 21: Signal Variability of the Aptima CV/TV Assay by Panel Member

Panel Description	N	Mean TTime	Between Days		Between Instruments		Between Operators		Between Lots		Between Runs		Within Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
C. albicans Low Positive	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
C. glabrata Low Positive	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
TV Low Positive	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
C. albicans Moderate Positive	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
C. glabrata Moderate Positive	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
TV Moderate Positive	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 = threshold time.

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.

Co-Infection

A co-infection study evaluated the ability of the Aptima CV/TV assay to detect C spp, *C. glabrata,* and TV when more than one organism is present in the same specimen. Low concentration of one target lysate and high concentration of another target lysate in SVSM were tested in combination. Panel composition and concentrations are listed in Table 22. All testing resulted in 100% detection for both targets present except for the combination of low *C. glabrata* (3X LoD) and high TV ($1x10^4$ cells/mL or $1x10^5$ cells/mL). Further testing was conducted and resulted in 100% detection for the combination of low *C. glabrata* (3X LoD) and high TV ($1x10^3$ cells/mL).

Table 22: Co-Infection Panel

Panel Member	*C. albicans* Concentration	*C. glabrata* Concentration	TV Concentration
C. albicans Low; *C. glabrata* High	13317 CFU/mL[1]	$1x10^6$ CFU/mL	N/A
C. albicans Low; TV High	13317 CFU/mL[1]	N/A	$1x10^5$ cells/mL
C. glabrata Low; TV *High*	N/A	123 CFU/mL[2]	$1x10^3$ cells/mL
C. albicans High; *C. glabrata* Low	$1x10^6$ CFU/mL	123 CFU/mL[2]	N/A
C. albicans High; TV Low	$1x10^6$ CFU/mL	N/A	0.0072 cells/mL[3]
C. glabrata High; TV Low	N/A	$1x10^6$ CFU/mL	0.0072 cells/mL[3]

CFU = colony forming units.

[1] 3X LoD C. albicans.

[2] 3X LoD C. glabrata.

[3] 3X LoD TV.

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Serious incidents occurring in relation to the device in the European Union should be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

AW-31482-001 rev. 001

2024-12

Revision History	Date	Description
AW-31842-REG rev. 002	December 2024	• Revised a global, IVDR-compliant Aptima CV/TV assay IFU AW-31482-REG from rev. 001 to rev. 002 to provide clarification to the Specimen Handling subsection. • Implemented routine administrative updates.
AW-31842-001 rev. 001	December 2024	• Created a global, IVDR-compliant Aptima CV/TV assay IFU AW-31481 rev. 001 based on AW-31481-REG rev. 002 to provide commercialization support for a 250-test kit (Cat. No. PRD-07662). • Implemented routine administrative updates.