Aptima® Trichomonas vaginalis Assay (Panther® System)

For *in vitro* diagnostic use.

Rx only

General Information

Intended Use

The Aptima Trichomonas vaginalis (TV) assay is an *in vitro* qualitative nucleic acid amplification test (NAAT) for the detection of ribosomal RNA (rRNA) from *Trichomonas vaginalis* to aid in the diagnosis of trichomoniasis using the Panther® System.

The assay may be used to test the following specimens from symptomatic or asymptomatic individuals: clinician-collected endocervical swabs, clinician-collected and patient-collected vaginal swabs (in a clinical setting), female and male urine, and specimens collected in PreservCyt® Solution.

Summary and Explanation of the Test

Trichomonas vaginalis (*T. vaginalis*) is the most common curable sexually transmitted disease (STI) agent in the United States, with an estimated 6.9 million new cases occurring annually (1).

T. vaginalis infections in women cause vaginitis, urethritis, and cervicitis. Discharge and small hemorrhagic lesions may be present in the genitourinary tract. Complications can include premature labor, low-birth-weight offspring, premature rupture of membranes, and post-abortion or post-hysterectomy infection. An association with pelvic inflammatory disease, tubal infertility, and cervical cancer with previous episodes of trichomoniasis has been reported. Symptomatic women with trichomoniasis usually report of vaginal discharge, vulvovaginal soreness, and/or irritation. Dysuria is also common. However, it has been estimated that 10 to 50% of *T. vaginalis* infections in women are asymptomatic, and in men the proportion may even be higher (2, 3).

Reported symptoms of trichomonas urogenital tract infection in men include penile discharge, pain during urination and intercourse, and groin and testes pain (5). Prevalence of trichomonas infection in males ranges from 0.49% in a low-risk asymptomatic population (6) to 6% in populations at high risk for infection (7, 8).

Detection of *T. vaginalis* with traditional culture methods is technically challenging and requires up to 7 days. Immediate inoculation into the media is preferred, and proper incubation conditions are required in addition to frequent microscopic examinations of the media to successfully culture the protozoa. The sensitivity of culture has been estimated to range from 38% to 82% when compared to molecular methods due to problems visualizing low numbers of the organisms or the motility of the protozoa (4, 9).

T. vaginalis may also be detected using “wet-mount” preparation by mixing vaginal secretions with saline on a slide and examining the slide under a microscope. However, the wet-mount method is only 35% to 80% sensitive compared with culture (9). The sensitivity of the wet-mount method is highly dependent on the experience of the microscopist as well as the time of specimen transport to the laboratory.

Principles of the Procedure

The Aptima TV assay involves transcription-mediated amplification (Hologic® TMA), and hybridization protection assay (HPA) technologies.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the Aptima TV assay is performed in the laboratory, the target rRNA is

isolated from the specimens using a specific capture oligomer and magnetic microparticles in a method called target capture. The capture oligomer contains a sequence complementary to a specific region of the target molecule as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific region of the capture oligomer binds to a specific region of the target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecule bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Hologic TMA reaction amplifies a specific region of the small ribosomal subunit from *T. vaginalis* via DNA and RNA intermediates and generates RNA amplicon molecules. Detection of the rRNA amplification product sequences is achieved using nucleic acid-based hybridization protection assay (HPA). A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer and are reported as Relative Light Units (RLU).

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. For additional specific warnings and precautions, refer to the *Panther/Panther Fusion System Operator's Manual*.

D. 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.

E. Only personnel adequately trained in the use of the Aptima TV assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

F. For additional specific warnings, precautions and procedures to control contamination, consult the *Panther/Panther Fusion System Operator's Manual*.

Laboratory Related

G. Use only supplied or specified disposable laboratory ware.

H. 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.

I. **Warning: Irritant and Corrosive.** Avoid contact of Auto Detect 2 with skin, eyes and mucous membranes. If this fluid comes into contact with skin or eyes, wash with water. If this fluid spills, dilute the spill with water before wiping dry.

J. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution.

K. Dispose of all materials that have come in contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

L. 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

M. This assay has been tested using endocervical and vaginal swab specimens, female and male urine specimens, and PreservCyt solution liquid Pap specimens only. Performance with specimens other than those specified under Specimen Collection and Storage has not been evaluated.

N. 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.

O. After urine has been added in the urine transport tube, the liquid level must fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.

P. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.

Q. 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.

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

S. Upon piercing, liquid can discharge from Aptima transfer tube caps under certain conditions. Refer to the appropriate *Test Procedure* for more information.

T. 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.

U. If the lab receives a swab specimen transport tube with no swab, two swabs, a cleaning swab, or a swab not supplied by Hologic, the specimen must be rejected.

V. 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

W. 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.

X. Use Universal Precautions when handling controls.

Y. Avoid microbial and ribonuclease contamination of reagents.

Z. Do not use a kit or control after its expiration date.

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

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

AC. Some reagents in this kit are labeled with risk and safety symbols.

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the 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.*

Selection Reagent
Boric Acid 1 - 5%

WARNING
H315 - Causes skin irritation
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P337 + P313 - If eye irritation persists: Get medical advice/attention
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P332 + P313 - If skin irritation occurs: Get medical advice/attention
P362 - Take off contaminated clothing and wash before reuse

Reagent Storage and Handling Requirements

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

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2ºC to 8ºC	N/A	N/A
Enzyme Reagent	2ºC to 8ºC	N/A	N/A
Probe Reagent	2ºC to 8ºC	N/A	N/A
Target Capture Reagent B	2ºC to 8ºC	N/A	N/A
Amplification Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Enzyme Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Probe Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Selection Reagent	2ºC to 30ºC	2ºC to 30ºC	60 days
Target Capture Reagent	15ºC to 30ºC	15ºC to 30ºC	60 days
Positive Control	2ºC to 8ºC	N/A	Single Use Vial
Negative Control	2ºC to 8ºC	N/A	Single Use Vial
Ready Made Amplification Reagent	2ºC to 8ºC	2ºC to 8ºC	60 days
Ready Made Enzyme Reagent	2ºC to 8ºC	2ºC to 8ºC	60 days
Ready Made Probe Reagent	2ºC to 8ºC	2ºC to 8ºC	60 days

B. If the Selection Reagent is stored refrigerated, let it come to room temperature before placing on the Panther system.

C. Working Target Capture Reagent (wTCR) is stable for 60 days when stored at 15ºC to 30ºC. Do not refrigerate.

D. After reconstitution, the Amplification Reagent, Enzyme Reagent, and Probe Reagent are stable for 60 days when stored at 2ºC to 8ºC.

E. Discard any unused reconstituted reagents, wTCR, and Ready Made Reagents after 60 days, or after the Master Lot expiration date, whichever comes first.

F. Controls are stable until the date indicated on the vials.

G. Reagents stored on-board the Panther system have 72 hours of on-board stability.

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

I. The Probe Reagent, Reconstituted Probe Reagent and Ready Made Probe Reagent are photosensitive. Store the reagents protected from light.

J. 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.*

The Aptima TV assay is designed to detect the presence of *T. vaginalis* in clinician-collected endocervical and vaginal swab specimens, patient-collected vaginal swab specimens, female and male urine specimens, and PreservCyt solution liquid Pap specimens. Performance with specimens other than those collected with the following specimen collection kits has not been evaluated:

- Aptima® Multitest Swab Specimen Collection Kit
- Aptima Urine Collection Kit for Male and Female Urine Specimens
- Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- Aptima Specimen Transfer Kit (for use with gynecological samples collected in PreservCyt solution prior to cytology processing)

A. Specimen Collection

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

B. Specimen Transport and Storage Before Testing

1. Urogenital Swab Specimens
   a. After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested.
   b. Specimens must be assayed within 60 days of collection. If longer storage is needed, freeze the specimen transport tube at ≤ –20°C for up to 24 months.
2. Urine Specimens
   a. Urine specimens that are still in the primary collection container must be transported to the lab at 2°C to 30°C. Transfer the urine specimen into the Aptima urine specimen transport tube within 24 hours of collection.
   b. Store processed urine specimens at 2°C to 30°C and assay within 30 days after transfer. If longer storage is needed, store the processed urine specimen at ≤–20°C for up to 24 months after transfer.
3. PreservCyt Solution Liquid Pap Specimens
   a. Transport and store the PreservCyt solution specimen at 2°C to 30°C for up to 30 days.
   b. Specimens collected in PreservCyt solution must be transferred into an Aptima specimen transfer tube according to the instructions in the Aptima Specimen Transfer kit and Aptima Transfer Solution package insert.
   c. After transfer to an Aptima specimen transfer tube, specimens may be stored an additional 14 days at 15°C to 30°C or 30 days at 2°C to 8°C.
   d. If longer storage is needed, the PreservCyt solution specimen or the PreservCyt solution liquid Pap specimen diluted into the specimen transfer tube may be stored at ≤–20°C for up to 24 months after transfer.

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. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 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 and international transportation regulations.*

Panther System

Reagents for the Aptima 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 Trichomonas vaginalis Assay Kit

250 tests (2 boxes and 1 Controls kit) (Cat. No. 303537)

100 tests (2 boxes and 1 Controls kit) (Cat. No. 303536)

Aptima Trichomonas vaginalis Assay Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity	
		250-test kit	100-test kit
A	**Amplification Reagent** *Primers and nucleotides dried in buffered solution containing < 5% bulking agent.*	1 vial	1 vial
E	**Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial	1 vial
P	**Probe Reagent** *Chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial	1 vial
TCR-B	**Target Capture Reagent B** *Buffered solution containing < 5% detergent.*	1 x 0.56 mL	1 x 0.30 mL

Aptima Trichomonas vaginalis Assay Room Temperature Box (Box 2 of 2)
(store at room temperature, 15°C to 30°C upon receipt)

Symbol	Component	Quantity	
		250-test kit	100-test kit
AR	**Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL	1 x 11.9 mL
ER	**Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL	1 x 6.3 mL
PR	**Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL	1 x 15.2 mL
S	**Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL	1 x 43.0 mL
TCR	**Target Capture Reagent** *Buffered solution containing capture oligomers 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 Trichomonas vaginalis Controls Kit
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
NC	**Negative Control** *Non-infectious non-target nucleic acid in a buffered solution containing < 5% detergent.*	5 x 1.7 mL
PC	**Positive Control** *Non-infectious Trichomonas vaginalis organisms in buffered solution containing < 5% detergent.*	5 x 1.7 mL

Aptima Trichomonas vaginalis Ready Made Reagent Assay Kit,
250 tests (2 boxes and 1 Controls kit) (Cat. No. PRD-07041)

Aptima Trichomonas vaginalis Ready Made Reagent Assay Refrigerated Box (Box 1 of 2) (store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
AR	**Ready Made Amplification Reagent** *Aqueous solution containing preservatives and nucleic acids.*	1 x 27.7 mL
ER	**Ready Made Enzyme Reagent** *HEPES buffered solution containing enzyme.*	1 x 11.1 mL
PR	**Ready Made Probe Reagent** *Succinate buffered solution containing chemiluminescent DNA probes.*	1 x 35.4 mL
TCR-B	**Target Capture Reagent B** *Non-infectious nucleic acids in buffered solution containing < 5% detergent.*	1 x 0.56 mL

Aptima Trichomonas vaginalis Ready Made Reagent Assay Room Temperature Box (Box 2 of 2) (store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
S	**Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL
TCR	**Target Capture Reagent** *Buffered solution containing capture oligomers and magnetic particles.*	1 x 54.0 mL
	Master Lot Barcode Sheet	1 sheet

Aptima Trichomonas vaginalis Controls Kit (store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
NC	**Negative Control** *Non-infectious non-target nucleic acid in a buffered solution containing < 5% detergent.*	5 x 1.7 mL
PC	**Positive Control** *Non-infectious Trichomonas vaginalis organisms in buffered solution containing < 5% detergent.*	5 x 1.7 mL

Materials Required But Available Separately

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

	Cat. No.
Panther System	303095
Panther System Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
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 Specimen Transfer Kit *for use with specimens in PreservCyt Solution*	301154C
Aptima Specimen Transfer Kit — printable *for use with specimens in PreservCyt Solution*	PRD-05110
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Urine Specimen Collection Kit for Male and Female Urine Specimens	301040
Aptima Urine Specimen Transport Tubes for Male and Female Urine Specimens	105575
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable gloves	—
Aptima penetrable caps	105668
Replacement non-penetrable caps	103036A
Replacement Caps for the 250-test kits	—
Amplification and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
Enzyme Reagent reconstitution solution	*501616 (100 caps)*
TCR and Selection reagent	*CL0040 (100 caps)*

Replacement Caps for 100-test kits		—
Amplification, Enzyme, and Probe reagent reconstitution solutions	*CL0041 (100 caps)*	
TCR and Selection reagent	*501604 (100 caps)*	

Optional Materials

	Cat. No.
Aptima Trichomonas vaginalis Controls Kit	302807
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 and samples 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 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.

B. Reagent Reconstitution/Preparation of a New Kit

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

Note: *For Ready Made Reagent Kits, skip to Step 2.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. 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).
   d. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 1, Step 2).

f. Slowly invert the assembled bottles. Allow the solution to drain from the reconstitution solution bottle into the glass vial (Figure 1, Step 3).

g. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (Figure 1, Step 4).

h. Wait 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 reconstitution solution bottle.

i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

j. Recap the reconstitution solution bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

k. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

Option: Additional mixing of the Amplification, Enzyme and Probe Reagents is allowed by placing 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.*

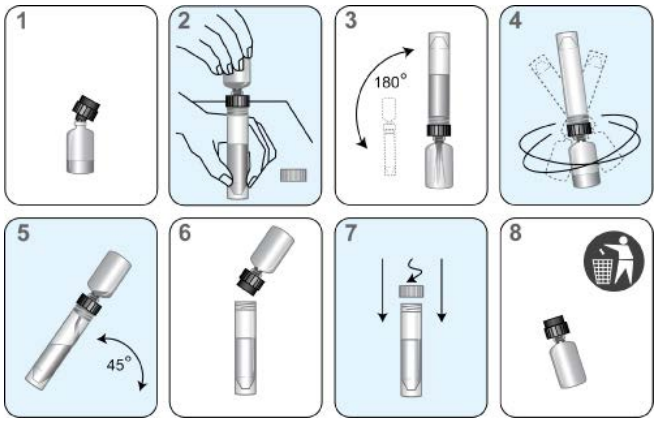

Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

Note: *Ready Made Reagent Assay Kits require wTCR preparation.*

a. Pair the appropriate bottles of TCR and TCR-B.

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 TCR-B and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the TCR-B bottle.

e. Cap the bottle of TCR 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 TCR-B bottle and cap.

3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure that it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents or Ready Made Reagents

Note: *Ready Made Reagent Assay Kits require wTCR preparation.*

1. For Ready Made Reagents, check the lot numbers on the Ready Made Amplification, Enzyme, and Probe Reagents to make sure they match the lot numbers on the Master Lot Barcode Sheet.
2. Previously reconstituted or Ready Made Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

   Option: The reconstituted or Ready Made Amplification, Enzyme, and Probe 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.

3. If reconstituted or Ready Made Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
4. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
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. Specimen Handling

1. Allow the controls and specimens to reach room temperature prior to processing.
2. Do not vortex specimens.
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single pink Aptima collection swab in a multitest swab specimen transport tube.
   b. A final volume of urine between the black fill lines of a urine specimen transport tube.
   c. The presence of a single blue Aptima collection swab in a unisex swab specimen transport tube.
   d. The absence of a swab in the Aptima specimen transport tube for PreservCyt solution liquid Pap specimens.

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.
   c. If the liquid level in a urine specimen tube is not between the two black indicator lines on the label, the specimen must be rejected. Do not pierce an overfilled tube.
   d. If a urine specimen tube contains precipitate, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, visually ensure that the precipitate does not prevent delivery of the specimen.

Note: *Failure to follow Steps 4a–4c 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* for the Panther system.

      Note: *Make sure that the appropriately sized reagent racks and TCR adapters are used.*
   2. Load samples.

Procedural Notes

A. Controls
   1. To work properly with the Aptima assay software on the Panther system, one pair of controls is required. The Positive Control and Negative Control can be loaded in any rack position or in any Sample Bay Lane on the Panther system. Patient specimen pipetting will begin when one of the following two conditions has been met:
      a. A pair of controls is currently being processed by the system.
      b. Valid results for the controls are registered on the system.
   2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours **unless:**
      a. Controls 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 Aptima control tube can be tested once. Attempts to pipette more than once from the tube 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.

D. 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 unisex swab specimen collection kit for endocervical and male urethral swab specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the Aptima Specimen Transport Medium (STM), 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 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 — QC/Patient Results*. For additional Panther system-specific contamination monitoring information, contact Hologic Technical Support.

Test Interpretation — QC/Patient Results

A. Test Interpretation

Assay test results are automatically interpreted by the Panther system Aptima TV assay software. A test result may be negative, positive, or invalid as determined by total RLU in the detection step (see below). A test result may be invalid due to RLU values outside the normal expected ranges. Initial invalid test results should be retested. Report the first valid result.

Test Interpretation	Total RLU (x1000)
Negative	0* to < 100
Positive	100 to < 2400
Invalid	0* or ≥ 2400

*If the RLU measured on the Panther System is between 0 and 999, a result of "0" is reported in the "Total RLU (000s)" column in the run report. Measured RLU values less than 690 are reported as invalid. RLU values between 690 and 999 are reported as valid.

B. Quality Control Results and Acceptability

The Negative Control, which is labeled "NC CONTROL – TRICH," and the Positive Control, which is labeled "PC CONTROL + TRICH," act as controls for the target capture, amplification, and detection steps of the assay. In accordance with guidelines or requirements of national, regional, and/or local regulations or accrediting organizations, additional controls for cell lysis and RNA stabilization may be included. The Positive Control, which is labeled "PC CONTROL + TRICH" contains non-infectious *T. vaginalis* rRNA.

The Controls must produce the following test results:

Control	Total RLU (x1000)	*T. vaginalis* Result
NC Control – TRICH	0* and < 20	Negative
PC Control + TRICH	≥ 500 and < 2400	Positive

*If the RLU measured on the Panther System is between 0 and 999, a result of "0" is reported in the "Total RLU (000s)" column in the run report. Measured RLU values less than 690 are reported as invalid. RLU values between 690 and 999 are reported as valid.

Note: Each laboratory should implement appropriate control procedures to satisfy the requirements of CLIA regulations.

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 insert may result in erroneous results.

B. The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of *T. vaginalis*.

C. TV-positive mucoid samples may exhibit decreased RLU values. To ensure proper endocervical sampling, excess mucus should be removed.

D. Urine, vaginal swab and PreservCyt solution liquid Pap specimen sampling is not designed to replace cervical exams and endocervical specimens for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.

E. This assay has been tested using only the specimen types indicated. Performance with other specimen types has not been evaluated.

F. Reliable results are dependent on adequate specimen collection. 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.

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

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

I. 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.

J. A negative result does not preclude a possible infection because the presence of *Trichomonas tenax* or *Pentatrichomonas hominis* in a specimen may affect the ability to detect *T. vaginalis* rRNA. See *Cross-Reactivity in the Presence of Microorganisms* for details.

K. Interference with the Aptima TV assay was observed in the presence of the following substances: Astroglide personal lubricant, mucus, and glacial acetic acid.

L. The Aptima 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.

M. The Aptima TV assay has not been validated for use with vaginal swab specimens collected by patients.

N. Performance has not been evaluated in adolescents less than 14 years of age.

O. The performance of the Panther system has not been determined at altitudes above 6561 feet (2000 m).

P. If a specimen has a small number of *T. vaginalis* organisms, uneven distribution of these trichomonads may occur, which may affect the ability to detect *T. vaginalis* rRNA in the collected material. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary.

Q. First-catch female urine specimens are acceptable for the detection of TV infections but may detect fewer TV infections when compared with vaginal swab specimens.

R. Customers must independently validate an LIS transfer process.

S. Aptima TV assay performance has not been evaluated with post-cytology processed specimens collected in PreservCyt solution.

Panther System Expected Values

Estimates of *T. vaginalis* in different populations depend on the sensitivity of the test in detecting the infection and on patient risk factors such as age, lifestyle, and the presence or absence of symptoms.

A summary of the positivity of *T. vaginalis* as determined by the Aptima TV assay during two muliticenter clinical studies, is shown in Table 1 and Table 2 for each specimen type and stratified by clinical site.

Table 1: Positivity of T. vaginalis as Determined by the Aptima TV Assay in Clinician-collected Vaginal Swab, Endocervical Swab, and PreservCyt Solution Liquid Pap Samples by Clinical Site (Clinical Study 1)

Site	Positivity % (# positive / # tested with valid results)		
	CVS	ES	PCyt
1	17.0 (9/53)	20.4 (11/54)	18.3 (11/60)
2	7.7 (4/52)	8.9 (5/56)	7.4 (5/68)
3	16.7 (2/12)	12.5 (2/16)	17.6 (3/17
4	19.5 (8/41)	17.1 (7/41)	18.6 (8/43)
5	0.7 (1/145)	0.6 (1/162)	0.6 (1/167)
6	16.0 (12/75)	20.2 (18/89)	22.1 (23/104)
7	12.0 (21/175)	9.1 (15/164)	11.2 (22/197)
8	15.0 (12/80)	13.3 (11/83)	10.5 (9/86)
9	24.4 (11/45)	20.8 (10/48)	22.9 (11/48)
ALL	11.8 (80/678)	11.2 (80/713)	11.8 (93/790)

CVS = clinician-collected vaginal swab; **ES** = endocervical swab; **PCyt** = PreservCyt Solution liquid Pap.

Table 2: Positivity of T. vaginalis as Determined by the Aptima TV Assay in Patient-collected Vaginal Swab, Female Urine, and Male Urine Samples by Clinical Site (Clinical Study 2)

Site	Positivity % (# positive / # tested with valid results)		
	PVS	FU	MU
1	0 (0/16)	0 (0/16)	0 (0/180)
2	11.1 (36/325)	10.4 (38/364)	4.4 (16/364)
3	8.5 (6/71)	9.5 (7/74)	1.7 (1/60)
4	NC (0/0)	NC (0/0)	0 (0/13)
5	8.8 (15/170)	8.8 (15/171)	2.9 (12/407)
6	5.8 (24/416)	5.8 (24/413)	0.7 (2/304)
7	6.1 (11/179)	5.3 (10/187)	1.3 (3/225)
8	0 (0/38)	0 (0/39)	0 (0/32)
9	10.8 (32/297)	9.8 (25/255)	2.4 (5/210)
10	20.2 (37/183)	19.8 (36/182)	6.7 (6/89)
11	6.7 (6/90)	3.7 (3/81)	0 (0/51)
ALL	9.4 (167/1785)	8.9 (158/1782)	2.3 (45/1935)

FU = female urine; **MU** = male urine; **NC** = not calculable; **PVS** = patient-collected vaginal swab.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive predictive value (PPV) and negative predictive value (NPV) of the Aptima TV assay across different hypothetical prevalence rates are shown for each specimen type in Table 3. These calculations are based on the overall estimated sensitivity and specificity for each specimen type from two multicenter clinical studies (See Table 4 and Table 5).

Table 3: Hypothetical PPV and NPV of the Aptima TV Assay by Specimen Type

Specimen Type	Prevalence (%)	PPV (%)	NPV (%)
CVS	1	35.4	100
	2	52.6	100
	5	74.1	100
	10	85.8	100
	15	90.6	100
	20	93.1	100
	25	94.8	100
PVS	1	64.3	100
	2	78.4	100
	5	90.4	99.9
	10	95.2	99.9
	15	96.9	99.8
	20	97.8	99.7
	25	98.3	99.6
ES	1	34.8	100
	2	51.8	100
	5	73.5	100
	10	85.4	100
	15	90.3	100
	20	93.0	100
	25	94.6	100
PCyt	1	41.1	100
	2	58.5	100
	5	78.4	100
	10	88.5	100
	15	92.4	100
	20	94.5	100
	25	95.8	100
MU	1	86.4	100
	2	92.8	100
	5	97.1	100
	10	98.6	100
	15	99.1	100
	20	99.4	100
	25	99.5	100

CVS = clinician-collected vaginal swab; **ES** = endocervical swab; **MU** = male urine; **PCyt** = PreservCyt Solution liquid Pap; **PVS** = patient-collected vaginal swab; **PPV** = positive predictive value; **NPV** = negative predictive value.

Panther System Clinical Performance

Two clinical studies were performed. Aptima TV assay clinical performance was estimated with clinician-collected vaginal swab, endocervical swab, and PreservCyt solution liquid pap specimens in Clinical Study 1, and with patient-collected vaginal swab, and female and male urine specimens in Clinical Study 2.

Clinical Study 1. Clinician-collected Vaginal Swab, Female Endocervical Swab, and PreservCyt Solution Liquid Pap Clinical Study

Clinical performance of the Aptima TV assay on the Panther system was evaluated using leftover specimens collected from consenting subjects during a previous, prospective, multicenter clinical study of the Aptima TV assay on the Tigris® DTS® System. Symptomatic and asymptomatic women were enrolled from 9 US clinical sites, including obstetrics and gynecology, family planning, and STD clinics. Three (3) vaginal swab, 1 endocervical swab, and 1 PreservCyt solution liquid Pap specimen were collected from each subject. All specimens were clinician-collected. All specimens were clinician-collected. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms.

PreservCyt liquid Pap specimens were collected with a broom-type device or a spatula and cytobrush. Two of the vaginal swab specimens were tested with a commercially available culture system and wet mount microscopic examination to establish infected status. The remaining specimens were prepared for Aptima TV assay testing in accordance with the appropriate Aptima specimen collection kit package insert instructions.

Panther system testing with the Aptima TV assay was conducted at 3 sites (2 external laboratories and Hologic) in accordance with package insert instructions.

Performance characteristics of the Aptima TV assay were estimated by comparing results to a patient infected status algorithm. In the algorithm, the designation of a subject as being infected or non-infected with *T. vaginalis* was based on results from vaginal swab specimens tested by culture and/or wet mount microscopic examination. At least one of the reference test results was required to be positive to establish an infected patient status. Both reference tests were required to be negative to establish a non-infected patient status.

Twenty-three (23) Aptima TV assay runs were initiated on the Panther system. Of these 23 runs, 1 (4.3%, 1/23) was aborted due to a fatal hardware error that led to a software failure. Specimens tested in the aborted run were retested. A total of 689 vaginal swab, 737 endocervical swab, and 791 PreservCyt solution liquid Pap specimens were tested in the 22 valid runs. Of these specimens, 12 vaginal swab (1.7%, 12/689), 24 endocervical swab (3.3%, 24/737), and 29 PreservCyt solution liquid Pap (3.7%, 29/791) specimens had initial invalid results due to hardware or software errors. Specimens with initial invalid results were retested. Eleven (11) vaginal swab (1.6%, 11/689), 24 endocervical swab (3.3%, 24/737), and 1 PreservCyt solution liquid Pap (0.1%, 1/791) specimens had final invalid results due to hardware or software errors; these specimens were excluded from the analyses.

Clinical Study 2. Patient-collected Vaginal Swab, and Female and Male Urine Clinical Study

The clinical performance of the Aptima TV assay on the Panther system was evaluated using specimens collected from consenting subjects in a prospective, multicenter clinical study.

Symptomatic and asymptomatic men and women were enrolled at 11 geographically and ethnically diverse US clinical sites, including obstetrics and gynecology, family planning, and STI clinics. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms.

Up to 5 specimens were collected from each female subject (4 patient-collected vaginal swabs, 1 first-catch urine), and 1 first catch urine specimen was collected from each male subject. All specimens were collected by the subject at the clinical sites.

Specimens were tested with the Aptima TV assay on the Panther system. Samples with initial invalid Aptima TV assay results were retested, volume permitting. Of the specimens collected, 5922 were processed in valid Aptima TV assay runs. Of these, 225 (3.8%, 95% CI: 3.3%–4.3%) had initial invalid results. Upon retest, 89 (1.5%) remained invalid and were excluded from the analyses. Urine and vaginal swabs were tested with up to three cleared NAATs to establish the specimen-specific composite comparator method result as follows:

- Male urine: The patient infected status (PIS) was derived from male urine specimens.
- Female urine: The composite comparator algorithm (CCA) interpretation was derived from female urine specimens.
- Vaginal swab: The PIS was derived from patient-collected vaginal swab specimens.

Specimens were categorized as infected (PIS) or positive (CCA) if a positive result occurred in at least two of the comparator NAATs, and as not infected or negative if at least 2 of the comparator results were negative; the third (tie-breaker) comparator was only required if the first 2 comparator results were discordant. Specimens that could not be categorized, due to missing results from the comparator assays, were excluded from the performance analyses. Performance of the Aptima TV assay was estimated relative to the PIS and reported as sensitivity and specificity for vaginal swab and male urine specimens, and was estimated relative to the CCA and reported as positive and negative percent agreement (PPA and NPA) for female urine specimens.

A total of 5502 specimens from 3820 evaluable subjects were included in the analyses comparing Aptima TV assay results to the specimen-specific PIS or CCA interpretations: 1785 patient-collected vaginal swab specimens, 1782 female urine specimens, and 1935 male urine specimens.

Performance Results

Performance characteristics of the Aptima TV assay were estimated for each specimen type and are displayed in Table 4, Table 5, and Table 6, including data from the two clinical studies. The infected status algorithm differed among the two studies. Table 4 shows the sensitivity, specificity, PPV, and NPV of the Aptima TV assay on the Panther system and the prevalence of *T. vaginalis* (based on the infected status) by symptom status and overall in female clinician-collected vaginal swab, endocervical swab, and PreservCyt solution liquid Pap specimens. Table 5 shows the sensitivity, specificity, PPV, and NPV of the assay in female patient-collected vaginal swab and male urine specimens based on the PIS. Table 6 shows the PPA and NPA of the assay in female urine specimens based on specimen-specific CCA. Prevalence was higher in symptomatic subjects.

Table 4: Performance Characteristics of the Aptima TV Assay in Clinician-collected Vaginal Swab, Endocervical Swab, and PreservCyt Liquid Pap Specimens by Symptom Status Compared to Patient Infected Status Algorithm (Clinical Study 1)

Specimen Type[1]	Symptom Status	n	TP	FP[2]	TN	FN	Prev %	Sensitivity % (95% CI)[3]	Specificity % (95% CI)[3]	PPV % (95% CI)[3,4]	NPV % (95% CI)[4]
CVS	Asymptomatic	274	12	7[a]	255	0	4.4	100 (75.8–100)	97.3 (94.6–98.7)	63.2 (45.8–80.9)	100 (98.8–100)
	Symptomatic	393	57	4[b]	332	0	14.5	100 (93.7–100)	98.8 (97.0–99.5)	93.4 (84.9–98.1)	100 (98.9–100)
	All	667	69	11[c]	587	0	10.3	100 (94.7–100)	98.2 (96.7–99.0)	86.3 (77.9–92.6)	100 (99.4–100)
ES	Asymptomatic	309	16	5[d]	288	0	5.2	100 (80.6–100)	98.3 (96.1–99.3)	76.2 (58.1–90.8)	100 (98.9–100)
	Symptomatic	391	51	7[e]	333	0	13.0	100 (93.0–100)	97.9 (95.8–99.0)	87.9 (78.1–94.7)	100 (99.0–100)
	All	700	67	12[f]	621	0	9.6	100 (94.6–100)	98.1 (96.7–98.9)	84.8 (76.3–91.5)	100 (99.4–100)
PCyt	Asymptomatic	333	19	2[g]	312	0	5.7	100 (83.2–100)	99.4 (97.7–99.8)	90.5 (72.6–98.7)	100 (98.9–100)
	Symptomatic	441	64	8[h]	369	0	14.5	100 (94.3–100)	97.9 (95.9–98.9)	88.9 (80.4–94.9)	100 (99.1–100)
	All	774	83	10[i]	681	0	10.7	100 (95.6–100)	98.6 (97.4–99.2)	89.2 (82.0–94.5)	100 (99.5–100)

CI = confidence interval; **CVS** = clinician-collected vaginal swab; **ES** = endocervical swab; **FN** = false negative; **FP** = false positive; **PCyt** = PreservCyt Solution liquid Pap; **Prev** = prevalence; **TN** = true negative; **TP** = true positive; **PPV** = positive predictive value; **NPV** = negative predictive value.

[1]Specimens were also tested by an alternative *T. vaginalis* NAAT assay with the following results (# positive results / # samples tested): [a]4/7; [b]3/4; [c]7/11; [d]1/5; [e]2/;7, [f]3/12; [g]0/2; [h]3/8; [i]3/10.

[2]Score confidence interval.

[3]PPV 95% confidence interval computed from the exact 95% confidence interval for the positive likelihood ratio, NPV 95% confidence interval computed from the exact 95% confidence interval from the negative likelihood ratio.

Table 5: Performance Characteristics of the Aptima TV Assay in Female Patient-collected Vaginal Swab, and Male Urine Specimens by Symptom Status Compared to PIS (Clinical Study 2)

Specimen Type[1]	Symptom Status	n	TP	FP[2]	TN	FN[3]	Prev %	Sensitivity % (95% CI)[4]	Specificity % (95% CI)[4]	PPV % (95% CI)[5]	NPV % (95% CI)[5]
PVS	Asym.	932	59	3[a]	868	2[a]	6.5	96.7 (88.8–99.1)	99.7 (99.0–99.9)	95.2 (89.5, 100)	99.8 (99.4, 100)
	Sym.	853	99	6[a]	748	0	11.6	100 (96.3–100)	99.2 (98.3–99.6)	94.3 (89.5, 98.2)	100 (NC)
	All	1785	158	9	1616	2	9.0	98.8 (95.6–99.7)	99.4 (99.0–99.7)	94.6 (91.1, 98.0)	99.9 (99.7, 100)
MU	Asym.	1125	21	1[b]	1103	0	1.9	100 (84.5–100)	99.9 (99.5–100)	95.5 (85.7, 100)	100 (NC)
	Sym.	810	21	2[c]	787	0	2.6	100 (84.5–100)	99.7 (99.1–99.9)	91.3 (78.6, 100)	100 (NC)
	All	1935	42	3	1890	0	2.2	100 (91.6–100)	99.8 (99.5–99.9)	93.3 (86.0, 100)	100 (NC)

CI = confidence interval; **Sym**. = symptomatic; **Asym**. = asymptomatic; **FN** = false negative; **FP** = false positive; **MU** = male urine; **NPV** = negative predictive value; **PPV** = positive predictive value; **Prev** = prevalence; **TN** = true negative; **TP** = true positive; **NC** = not calculable.

[1]All results are from Clinical Study 2.

[2]Volume permitting, samples of the same type, unless noted otherwise, were also tested by an alternative *T. vaginalis* NAAT assay with the following results (# positive results / # samples tested), [a]No discordant resolution testing result was available for PVS samples, [b]0/1, [c]0/1 (no discordant resolution testing result was available for 1 sample).

[3]Volume permitting, samples of the same type, unless noted otherwise, were also tested by an alternative *T. vaginalis* NAAT assay with the following results (# negative results / # samples tested): [a]No discordant resolution testing result was available for PVS samples.

[4]Score CI.

[5]Percentile CI obtained using the bootstrap re-sampling method with 2000 iterations. The statistic may be not calculable in some bootstrap samples due to a denominator of zero; the percentile CI is computed using the bootstrap samples in which the statistic can be calculated. If the statistic is not calculable in all bootstrap samples, or if the value of the statistic is constant across all bootstrap samples in which the statistic can be calculated, the 95% percentile bootstrap CI is set to NC.

Table 6: Performance Characteristics of the Aptima TV Assay in Female Urine Specimens by Symptom Status Compared to the CCA (Clinical Study 2)

Specimen Type[1]	Symptom Status	n	CCA+ ATV+	CCA- ATV+	CCA- ATV-	CCA+ ATV-	PPA % (95% CI)[2]	NPA % (95% CI)[2]
FU	Asymptomatic	949	64	0	885	0	100 (94.3–100)	100 (99.6–100)
	Symptomatic	833	94	0	739	0	100 (96.1–100)	100 (99.5–100)
	All	1782	158	0	1624	0	100 (97.6–100)	100 (99.8–100)

FU = female urine; **ATV** = Aptima TV Assay; **CCA** = composite comparator algorithm; **PPA** = positive percent agreement; **NPA** = negative percent agreement; **CI** = confidence interval.

[1]All results are from Clinical Study 2.

[2]Score CI.

The performance of the Aptima TV assay in female urine specimens was also assessed compared to a patient-collected vaginal swab-based PIS. The data shows that the detection of *T. vaginalis* infection in female urine specimens by the Aptima TV assay is up to 2.4% lower when using the vaginal swab PIS compared to the female urine CCA.

Table 7 shows the sensitivity, specificity, PPV, and NPV of the Aptima TV assay on the Panther system and the prevalence of *T. vaginalis* (based on the patient infected status algorithm) in female clinician-collected vaginal swab, endocervical swab, and by collection site (Clinical Study 1).

Table 8 shows the sensitivity, specificity, PPV, and NPV of the assay in female patient-collected vaginal swaband male urine specimens (Clinical Study 2). Table 9 shows the PPA and NPA of the assay in female urine specimens by collection site (Clinical Study 2).

Table 7: Performance Characteristics of the Aptima TV Assay by Collection Site (Clinical Study 1)

Site	Specimen Type[1]	n	TP	FP	TN	FN	Prev %	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
1	CVS	52	8	1	43	0	15.4	100 (67.6–100)	97.7 (88.2–99.6)	88.9 (60.2–99.7)	100 (93.7–100)
	ES	53	9	2	42	0	17.0	100 (70.1–100)	95.5 (84.9–98.7)	81.8 (56.9–97.4)	100 (93.5–100)
	PCyt	59	11	0	48	0	18.6	100 (74.1–100)	100 (92.6–100)	100 (75.6–100)	100 (93.9–100)
2	CVS	52	3	1	48	0	5.8	100 (43.9–100)	98.0 (89.3–99.6)	75.0 (28.5–99.2)	100 (95.8–100)
	ES	56	4	1	51	0	7.1	100 (51.0–100)	98.1 (89.9–99.7)	80.0 (40.5–99.4)	100 (95.6–100)
	PCyt	68	5	0	63	0	7.4	100 (56.6–100)	100 (94.3–100)	100 (58.3–100)	100 (96.0–100)
3	CVS	12	2	0	10	0	16.7	100 (34.2–100)	100 (72.2–100)	100 (32.1–100)	100 (85.6–100)
	ES	16	2	0	14	0	12.5	100 (34.2–100)	100 (78.5–100)	100 (31.5–100)	100 (89.3–100)
	PCyt	17	2	1	14	0	11.8	100 (34.2–100)	93.3 (70.2–98.8)	66.7 (19.9–98.8)	100 (89.5–100)
4	CVS	41	7	1	33	0	17.1	100 (64.6–100)	97.1 (85.1–99.5)	87.5 (57.3–99.6)	100 (92.2–100)
	ES	41	7	0	34	0	17.1	100 (64.6–100)	100 (89.8–100)	100 (66.7–100)	100 (92.2–100)
	PCyt	43	7	1	35	0	16.3	100 (64.6–100)	97.2 (85.8–99.5)	87.5 (57.2–99.6)	100 (92.6–100)
5	CVS	145	1	0	144	0	0.7	100 (20.7–100)	100 (97.4–100)	100 (6.4–100)	100 (99.3–100)
	ES	162	1	0	161	0	0.6	100 (20.7–100)	100 (97.7–100)	100 (6.4–100)	100 (99.4–100)
	PCyt	167	1	0	166	0	0.6	100 (20.7–100)	100 (97.7–100)	100 (6.4–100)	100 (99.4–100)
6	CVS	67	10	2	55	0	14.9	100 (72.2–100)	96.5 (88.1–99.0)	83.3 (59.2–98.2)	100 (94.8–100)
	ES	80	13	4	63	0	16.3	100 (77.2–100)	94.0 (85.6–97.7)	76.5 (57.1–92.2)	100 (95.3–100)
	PCyt	92	20	3	69	0	21.7	100 (83.9–100)	95.8 (88.5–98.6)	87.0 (70.4–97.0)	100 (95.5–100)
7	CVS	173	18	3	152	0	10.4	100 (82.4–100)	98.1 (94.5–99.3)	85.7 (67.7–96.7)	100 (97.9–100)
	ES	161	12	3	146	0	7.5	100 (75.8–100)	98.0 (94.2–99.3)	80.0 (58.3–95.4)	100 (97.9–100)
	PCyt	194	18	4	172	0	9.3	100 (82.4–100)	97.7 (94.3–99.1)	81.8 (64.1–94.3)	100 (98.1–100)

Table 7: Performance Characteristics of the Aptima TV Assay by Collection Site (Clinical Study 1) (continued)

Site	Specimen Type[1]	n	TP	FP	TN	FN	Prev %	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
8	CVS	80	10	2	68	0	12.5	100 (72.2–100)	97.1 (90.2–99.2)	83.3 (59.0–98.2)	100 (95.8–100)
	ES	83	9	2	72	0	10.8	100 (70.1–100)	97.3 (90.7–99.3)	81.8 (56.3–97.4)	100 (96.1–100)
	PCyt	86	9	0	77	0	10.5	100 (70.1–100)	100 (95.2–100)	100 (71.4–100)	100 (96.2–100)
9	CVS	45	10	1	34	0	22.2	100 (72.2–100)	97.1 (85.5–99.5)	90.9 (65.7–99.7)	100 (91.9–100)
	ES	48	10	0	38	0	20.8	100 (72.2–100)	100 (90.8–100)	100 (74.0–100)	100 (92.5–100)
	PCyt	48	10	1	37	0	20.8	100 (72.2–100)	97.4 (86.5–99.5)	90.9 (65.6–99.7)	100 (92.5–100)

CI = confidence interval; **CVS** = clinician-collected vaginal swab; **ES** = endocervical swab; **FN** = false negative; **FP** = false positive; **PCyt** = PreservCyt Solution liquid Pap; **Prev** = prevalence; **TN** = true negative; **TP** = true positive; **PPV** = positive predictive value; **NPV** = negative predictive value.

[1]All results are from Clinical Study 1.

[2]Score CI.

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

Table 8: Performance Characteristics of the Aptima TV Assay in Female Patient-collected Vaginal Swab and Male Urine Specimens by Collection Site, Compared to the PIS (Clinical Study 2)

Site	Specimen Type[1]	n	TP	FP	TN	FN	Prev %	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
1	PVS	16	0	0	16	0	0.0	NC	100 (80.6–100)	NC	100 (NC)
	MU	180	0	0	180	0	0.0	NC	100 (97.9–100)	NC	100 (NC)
2	PVS	325	31	5	288	1	9.8	96.9 (84.3–99.4)	98.3 (96.1–99.3)	86.1 (74.3, 97.0)	99.7 (98.9, 100)
	MU	364	16	0	348	0	4.4	100 (80.6–100)	100 (98.9–100)	100 (NC)	100 (NC)
3	PVS	71	6	0	65	0	8.5	100 (61.0–100)	100 (94.4–100)	100 (NC)	100 (NC)
	MU	60	1	0	59	0	17	100 (20.7–100)	100 (93.9–100)	100 (NC)	100 (NC)
4	PVS[3]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	MU	13	1	0	13	0	0.0	NC	100 (77.2–100)	NC	100 (NC)
5	PVS	170	15	0	155	0	8.8	100 (79.6–100)	100 (97.6–100)	100 (NC)	100 (NC)
	MU	407	11	1	395	0	2.7	100 (74.1–100)	99.7 (98.6–100)	91.7 (73.3, 100)	100 (NC)
6	PVS	416	24	0	392	0	5.8	100 (86.2–100)	100 (99.0–100)	100 (NC)	100 (NC)
	MU	304	2	0	302	0	0.7	100 (34.2–100)	100 (98.7–100)	100 (NC)	100 (NC)
7	PVS	179	11	0	168	0	6.1	100 (74.1–100)	100 (97.8–100)	100 (NC)	100 (NC)
	MU	225	2	1	222	0	0.9	100 (34.2–100)	99.6 (97.5–99.9)	66.7 (0.0, 100)	100 (NC)
8	PVS	38	0	0	38	0	0.0	NC	100 (90.8–100)	NC	100 (NC)
	MU	32	0	0	32	0	0.0	NC	100 (89.3–100)	NC	100 (NC)
9	PVS	297	28	4	265	0	9.4	100 (87.9–100)	98.5 (96.2–99.4)	87.5 (74.7, 97.2)	100 (NC)
	MU	210	5	0	205	0	2.4	100 (56.6–100)	100 (98.2–100)	100 (NC)	100 (NC)
10	PVS	183	37	0	145	1	20.8	97.4 (86.5–99.5)	100 (97.4–100)	100 (NC)	99.3 (97.9, 100)
	MU	89	5	1	83	0	5.6	100 (56.6–100)	98.8 (93.6–99.8)	83.3 (50.0, 100)	100 (NC)
11	PVS	90	6	0	84	0	6.7	100 (61.0–100)	100 (95.6–100)	100 (NC)	100 (NC)
	MU	51	0	0	51	0	0.0	NC	100 (93.0–100)	NC	100 (NC)

CI = confidence interval; **PVS** = patient-collected vaginal swab; **MU** = male urine; **FN** = false negative; **FP** = false positive; **Prev** = prevalence; **TN** = true negative; **TP** = true positive; **PPV** = positive predictive value; **NPV** = negative predictive value; **N/A** = not available; **NC** = not calculable.

[1]All results are from Clinical Study 2.

[2]Score CI.

[3]There were no female subjects evaluable for patient-collected vaginal swab specimens at this site.

Table 9: Performance Characteristics of the Aptima TV Assay in Female Urine Specimens by Collection Site, Compared to the CCA (Clinical Study 2)

Specimen Type[1]	Site	N	CCA+ ATV+	CCA- ATV+	CCA- ATV-	CCA+ ATV-	PPA % (95% CI)[2]	NPA % (95% CI)[2]
FU	1	16	0	0	16	0	NC	100 (80.6–100)
	2	364	38	0	326	0	100 (90.8–100)	100 (98.8–100)
	3	74	7	0	67	0	100 (64.6–100)	100 (94.6–100)
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	5	171	15	0	156	0	100 (79.6–100)	100 (97.6–100)
	6	413	24	0	389	0	100 (86.2–100)	100 (99.0–100)
	7	187	10	0	177	0	100 (72.2–100)	100 (97.9–100)
	8	39	0	0	39	0	NC	100 (91.0–100)
	9	255	25	0	230	0	100 (86.7–100)	100 (98.4–100)
	10	182	36	0	146	0	100 (98.4–100)	100 (97.4–100)
	11	81	3	0	78	0	100 (43.9-100)	100 (95.3-100)

FU = female urine; **ATV** = Aptima TV Assay; **CCA** = composite comparator algorithm; **PPA** = positive percent agreement; **NPA** = negative percent agreement; **CI** = confidence interval; **NC** = not calculable.

[1]All results are from Clinical Study 2.

[2]Score CI.

[3]There were no female subjects evaluable for female urine specimens at this site.

Table 10 shows the sensitivity, specificity, PPV, and NPV of the Aptima TV assay on the Panther system and the prevalence of *T. vaginalis* (based on the infected status) in PreservCyt solution liquid Pap specimens by cervical collection device. For PreservCyt solution liquid Pap specimens, performance was similar across collection devices.

Table 10: Performance Characteristics of the Aptima TV Assay in PreservCyt Solution Liquid Pap Specimens by Collection Device Type (Clinical Study 1)

Collection Device[1]	n	TP	FP	TN	FN	Prev %	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
Broom-type Device	414	54	5	355	0	13.0	100 (93.4–100)	98.6 (96.8–99.4)	91.5 (82.4–97.1)	100 (99.0–100)
Spatula/Cytobrush	360	29	5	326	0	8.1	100 (88.3–100)	98.5 (96.5–99.4)	85.3 (71.5–94.7)	100 (99.0–100)

CI = confidence interval; **FN** = false negative; **FP** = false positive; **Prev** = prevalence; **TN** = true negative; **TP** = true positive.

[1]All results are from Clinical Study 1.

[2]Score CI.

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

Agreement of Aptima TV Assay Results on the Panther System and the Tigris DTS System

It is recognized that device performance in an asymptomatic population is essential since the majority of individuals infected with *T. vaginalis* do not have symptoms. To further characterize performance of the assay in asymptomatic subjects, agreement between Aptima TV assay results on the Panther system and the Tigris DTS system was assessed using prospectively collected specimens from asymptomatic subjects. Women were enrolled from 6 US clinical sites, including obstetrics and gynecology, family planning, and STD clinics. One (1) vaginal swab, 1 endocervical swab, and 1 PreservCyt solution liquid Pap specimen were collected from each subject. All specimens were clinician-collected. PreservCyt solution liquid Pap specimens were collected with a broom-type device or a spatula and cytobrush.

Aptima TV assay testing was conducted in accordance with the package insert instructions. Panther system testing was conducted at 3 sites (2 external laboratories and Hologic). Tigris DTS system testing was conducted at Hologic.

Eighteen (18) Aptima TV assay runs were initiated on the Panther system; all were valid. A total of 227 vaginal swab, 227 endocervical swab, and 227 PreservCyt solution liquid Pap specimens were tested. Of these specimens, 1 vaginal swab specimen (0.4%, 1/227) had an initial invalid result due to hardware error. The specimen with an initial invalid result was retested and had a valid result.

Of the samples with final valid Aptima TV assay results on the Panther system, 227 vaginal swab, 227 endocervical swab, and 226 PreservCyt solution liquid Pap specimens had valid, paired results on the Tigris DTS system.

Table 11 shows positive and negative percent agreements of Aptima TV assay results on the Panther system and the Tigris DTS system in each specimen type for asymptomatic subjects from Clinical Study 1.

Table 11: Agreement between Aptima TV Assay Results on the Panther System and the Tigris DTS System in Asymptomatic Subjects (Clinical Study 1)

Specimen Type	n	System					% Positive Agreement (95% CI)[2]	% Negative Agreement (95% CI)[2]
		Tigris + Panther +	Tigris - Panther +	Tigris - Panther -	Tigris + Panther -	Tigris Positivity		
CVS[1]	227	29	5	191	2	13.7	93.5 (79.3–98.2)	97.4 (94.2–98.9)
ES	227	28	1	198	0	12.3	100 (87.9–100)	99.5 (97.2–99.9)
PCyt	226	26	1	199	0	11.5	100 (87.1–100)	99.5 (97.2–99.9)

+ = positive; - = negative; **CI** = confidence interval; **CVS** = clinician-collected vaginal swab; **ES** = endocervical swab; **PCyt** = PreservCyt Solution liquid Pap.

[1]The 2 vaginal swab samples with positive Aptima TV Assay results on the Tigris DTS System and negative results on the Panther System were from subjects whose other samples had negative results on both the Panther System and the Tigris DTS System.

[2]Score confidence interval.

RLU Distribution of Aptima TV Assay Controls

The distribution of the RLU values for the Aptima TV assay controls is presented in Table 12 for all valid Aptima TV assay runs performed during Clinical Study 1 and Clinical Study 2.

Table 12: RLU Distribution of Aptima TV Negative and Positive Controls

Control	Statistic	Total RLU (x1000)	
		Clinical Study 1	Clinical Study 2
Negative	N	22	155
	Mean	1.3	NC
	SD	0.99	NC
	Median	1.0	1.0
	Minimum	0	1
	Maximum	5	12
	CV%	75.5	91.60

Table 12: RLU Distribution of Aptima TV Negative and Positive Controls (continued)

Positive	N	22	155
	Mean	1262.3	NC
	SD	45.89	NC
	Median	1276.0	1400.0
	Minimum	1168	1157
	Maximum	1322	1612
	CV%	3.6	5.97

CV% = percent coefficient of variation; **NC** = not calculable; **RLU** = relative light units.
Note: The RLU value reported by the software was the basis for analysis. The reported RLU value is the total measured RLU divided by 1000 with the digits after the decimal point truncated.

Reproducibility Study

Aptima TV assay reproducibility was evaluated on the Panther system at two external US laboratories and at Hologic. Testing was performed using two lots of assay reagents and a total of six operators (two at each site). At each site, testing was performed over at least 6 days.

Reproducibility panel members were created by using negative urine specimens in Urine Transport Medium (UTM) or negative PreservCyt solution liquid Pap specimens with STM. The positive panel members were created by spiking the urine matrix or PreservCyt solution liquid Pap matrix with the appropriate amount of *T. vaginalis* lysate. Final *T. vaginalis* concentrations ranged from 0.002 trichomonads/mL to 1 trichomonads/mL.

Table 13 presents, for each panel member, RLU data in terms of mean, standard deviation (SD), and coefficient of variation (CV) between sites, between operators, between lots, between runs, within runs, and overall (Total). Percent agreement with expected results is also shown. Samples with valid results were included in the analyses.

Table 13: Aptima Trichomonas vaginalis Assay Reproducibility Study

Conc	N	Agmt (%)	Mean RLU	Between Sites		Between Operators		Between Lots		Between Runs		Within Runs		Totals	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
PreservCyt Solution Liquid Pap Matrix Samples															
Neg	108	99.1	23.5	0.0	0.0	2.7	11.6	0.0	0.0	0.0	0.0	37.5	159.7	37.6	160.1
HNeg	108	90.7	69.3	5.0	7.3	4.5	6.5	6.1	8.8	14.8	21.4	16.0	23.1	23.6	34.1
MPos	108	97.2	348.1	30.3	8.7	33.1	9.5	33.1	9.5	77.0	22.1	62.9	18.1	114.0	32.8
HPos	108	100	1185.5	0.0	0.0	17.0	1.4	0.0	0.0	28.0	2.4	34.2	2.9	47.4	4.0
Urine Matrix Samples															
Neg	108	100	1.0	0.2	24.6	0.0	0.0	0.3	28.3	0.0	0.0	0.7	72.3	0.8	81.4
HNeg	107	100	33.1	15.9	48.1	4.9	14.8	0.0	0.0	7.1	21.6	9.3	28.0	20.3	61.5
MPos	108	100	621.9	27.2	4.4	33.5	5.4	37.3	6.0	100.6	16.2	69.4	11.2	134.9	21.7
HPos	108	100	1208.3	28.8	2.4	0.0	0.0	0.0	0.0	140.4	11.6	41.5	3.4	149.2	12.3

Agmt = agreement; **Conc** = concentration; **CV (%)** = coefficient of variation; **HNeg** = high negative; **HPos** = high positive; **MPos** = moderate positive; **Neg** = negative; **RLU** = relative light units; **SD** = standard deviation.
Note: The RLU value reported by the software is the total measured RLU divided by 1000 with the digits after the decimal point truncated.
Variability from some factors may have been numerically negative. This occurred if the variability due to those factors was very small. In these cases, SD and CV are shown as 0.

Panther System Analytical Performance

Analytical Sensitivity

Sensitivity panels were prepared with two strains of *T. vaginalis* (one Metronidazole-susceptible strain and one Metronidazole-resistant strain). Testing showed greater than 95% positivity in both strains of *T. vaginalis* for panels containing 0.01 TV/mL in PreservCyt solution liquid Pap specimen matrix, panels containing 0.01 TV/mL in urine specimen matrix, and panels containing 0.003 TV/mL in swab specimen matrix.

Cross-Reactivity in the Presence of Microorganisms

Cross-reactivity

Potential cross-reactivity of the Aptima TV assay was evaluated by testing various microorganisms, including common flora of the genitourinary tract, opportunistic organisms, and closely related organisms. Testing was conducted in STM, urine, and PreservCyt solution in STM with 25 replicates of each isolate. The list of organisms and the concentrations tested are provided in Table 14. No cross-reactivity was observed with any of the organisms tested.

Microbial Interference

Potential for microbial interference with the Aptima TV assay was evaluated by testing the same organisms (Table 14) in STM, urine, and PreservCyt solution in STM spiked with *T. vaginalis* lysate to a final concentration of 0.01 TV/mL (25 replicates of each isolate). Positivity of the Aptima TV assay was not affected by the presence of the microorganisms tested, except in the presence of *Trichomonas tenax* and *Pentatrichomonas hominis*. *Trichomonas tenax* is a commensal of the oral cavity and *Pentatrichomonas hominis* and *Dientamoeba fragilis* are commensal of the large intestine.

Table 14: Microorganisms Tested in the Aptima Trichomonas vaginalis Assay

Microorganism	Concentration	Microorganism	Concentration
Acinetobacter lwoffi	$1x10^6$ CFU/mL	HPV 16	$2.5x10^6$ copies/mL
Actinomyces israelii	$1x10^6$ CFU/mL	HPV 6	$2.5x10^6$ copies/mL
Atopobium vaginae	$1x10^6$ CFU/mL	*Klebsiella pneumoniae*	$1x10^6$ CFU/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^6$ CFU/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Lactobacillus crispatus*	$1x10^6$ CFU/mL
Campylobacter jejuni	$1x10^6$ CFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Candida albicans	$1x10^6$ CFU/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Chlamydia trachomatis	$1x10^6$ IFU/mL	*Mycoplasma genitalium*	$2.5\ x10^6$ copies/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	*Pentatrichomonas hominis*	$1x10^6$ cells/mL
Cytomegalovirus	$2x10^5$ $TCID_{50}$/mL	*Peptostreptococcus magnus*	$1x10^6$ CFU/mL
Dientamoeba fragilis	$1x10^6$ CFU/mL	*Prevotella bivia*	$1x10^6$ CFU/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Propionibacterium acnes*	$1x10^6$ CFU/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Escherichia coli	$1x10^6$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Gardnerella vaginalis	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL

Table 14: Microorganisms Tested in the Aptima Trichomonas vaginalis Assay (continued)

Microorganism	Concentration	Microorganism	Concentration
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Herpes simplex virus I	$2x10^5$ $TCID_{50}$/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
Herpes simplex virus II	$2x10^5$ $TCID_{50}$/mL	*Trichomonas tenax*	$1x10^6$ cells/mL
HIV-1	$2.5x10^6$ copies/mL	*Ureaplasma urealyticum*	$1x10^6$ CFU/mL

Interfering Substances

The following substances (Table 15) were individually spiked into STM, urine in UTM, and PreservCyt solution in STM for a final concentration of 1% (vol/vol or wt/vol): personal lubricants, personal deodorants, spermicides, anti-fungals, intravaginal hormones, porcine gastric mucus, seminal fluid from 25 donors, and whole blood (10% final concentration). Glacial acetic acid was tested by spiking into PreservCyt solution-STM (10% final concentration). Samples with each interfering substance alone as well as samples spiked with *T. vaginalis* lysate to a final concentration of 0.01 TV/mL were tested.

Testing results yielded no false positive results for all substances tested. Lower mean RLU (<1,000,000) was observed with mucin at 1%. The mean RLU value was above 1,000,000 when testing samples with 0.5% mucin. *T. vaginalis* was detected at ≥ 95% in the presence of all substances tested with the exception of Astroglide personal lubricant, mucus, and glacial acetic acid.

Additional interference testing was conducted to evaluate the effect of Astroglide personal lubricant, mucus, and glacial acetic acid by spiking *T. vaginalis* at 0.3 and 1 TV/mL in STM, PreservCyt-STM and urine-UTM matrices. *T. vaginalis* at 0.3 TV/mL was detected at ≥ 95% in the presence of Astroglide personal lubricant. *T. vaginalis* at 1 TV/mL was detected at ≥ 95% in the presence of mucus and glacial acetic acid. Glacial acetic acid would only be present in PreservCyt specimens and not in urine or swab specimens.

Table 15: ATV Assay Interference Panel

Product Category	Concentration	V/V or W/V
Lubricant	1%	V/V
Spermicide	1%	W/V
Anti-fungal	1%	W/V
Deodorant Spray/Powder	1%	W/V
Intra-vaginal Hormones	1%	W/V
Seminal Fluid	1%	V/V
Mucus	1%	W/V
Whole Blood	10%	V/V
Glacial Acetic Acid	10%	V/V
Urine Metabolites	100% in place of urine	N/A

V/V = volume/volume; **W/V** = weight/volume; **N/A** = not applicable.

Carryover Studies for the Panther System

To establish that the Panther system minimizes the risk of false positive results arising from carryover contamination, a multi-day analytical study was conducted using spiked panels on

three Panther systems with one lot of Aptima TV assay reagents. The study used > 20% high-target *T. vaginalis* samples containing 10,000 TV/mL, which were placed among negative samples containing STM. Over the course of the study, 698 high-target samples and 2,266 negative samples were tested across the three Panther systems. There were 0 false positive results for a 0% carryover contamination rate. These results demonstrate that carryover contamination is minimized on the Panther system.

Specimen Stability

Data to support the recommended shipping and storage conditions for swab, urine, and PreservCyt solution liquid Pap specimens were generated with negative clinical specimens spiked with *T. vaginalis*. Greater than 97% positivity was observed in all matrices (swab, urine, and PreservCyt solution liquid Pap specimens) at all times and temperatures tested confirming the validity of the maximum storage times and temperatures described in *Specimen Collection and Storage*.

Bibliography

1. **Kreisel, K.M., et al.** 2018. Sexually transmitted infections among US women and men: Prevalence and incidence estimates, Sex. Transm. Dis. **48**(4):208-214.
2. **Sutton, M., M. Sternberg, E.H. Koumans, G. McQuillan, S. Berman, L. Markowitz.** 2007. The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States. Clin. Infect. Dis. **45**(10):1319-1326.
3. **. Seña, A.C., W.C. Miller, M.M. Hobbs, J.R. Schwebke, P.A. Leone, et. al.** 2007.*Trichomonas vaginalis* infection in male sexual partners: implications for diagnosis, treatment, and prevention. Clin. Infect. Dis. **44**(1):13-22.
4. **Nye, M. B., J.R. Schwebke, and B.A. Body.** 2009. Comparison of Aptima Trichomonas vaginalis transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. Am. J. Obstet. Gynecol. **200**:188.e1-188.e7.
5. **Gaydos C.A., M.R. Barnes, N. Quinn, M. Jett-Goheen Y.H. Hsieh.** 2013. *Trichomonas vaginalis* infection in men who submit self-collected penile swabs after internet recruitment. Sex. Transm. Infect. **89**(6):504-8.
6. **Daugherty M., K. Glynn, and T. Byler.** 2019. Prevalence of *Trichomonas vaginalis* Infection Among US Males. Clin. Infect. Dis. **68**(3):460-465.
7. **Munson K.L., M. Napierala, E. Munson, R.F. Schell, T. Kramme, C. Miller, J.E. Hryciuk.** 2013. Screening of male patients for *Trichomonas vaginalis* with transcription-mediated amplification in a community with a high prevalence of sexually transmitted infection. J. Clin. Microbiol. **51**(1):101-4.
8. **Schwebke J., A. Merriweather, S. Massingale, M. Scisney, C. Hill, D. Getman.** 2018**.** Screening for *Trichomonas vaginalis* in a Large High-Risk Population: Prevalence Among Men and Women Determined by Nucleic Acid Amplification Testing. Sex. Transm. Dis. **45**(5):e23-e24.
9. **Wendel, K.A., E.J. Erbelding, C.A. Gaydos, and A.M. Rompalo.** 2002. *Trichomonas vaginalis* polymerase chain reaction compared with standard diagnostic and therapeutic protocols for detection and treatment of vaginal trichomoniasis. Clin. Infect. Dis. **35**(5):576-580.

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