Aptima® Trichomonas vaginalis Assay (Panther® System)

Instructions for Use
For *in vitro* diagnostic use
For U.S. Export 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, female and male urine specimens, and specimens collected in PreservCyt® Solution.

Summary and Explanation of the Test

T. vaginalis (TV) is the most common curable sexually transmitted disease (STD) agent in the United States, with an estimated 7.4 million new cases occurring annually (1, 2).

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 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 (3, 4, 5).

Reported symptoms of trichomonas urogenital tract infection in men include penile discharge, pain during urination and intercourse, and groin and testes pain (6). Prevalence of trichomonas infection in males ranges from 0.49% in a low-risk asymptomatic population (7) to 6% in populations at high risk for infection (8, 9).

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 (10, 11).

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 (11). 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 the technologies of target capture, transcription-mediated amplification (TMA), and hybridization protection assay (HPA).

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 by 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).

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 Aptima TV assay, refer to the Basic Unique Device Identifier (BUDI), which is: **54200455DIAGAPTTRICHWY**.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther/Panther Fusion® System Operator's Manual* prior to performing the assay.

D. 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.

E. For additional specific warnings, precautions, and procedures to control contamination for the *Panther/Panther Fusion® 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. **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.

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

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

K. 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

L. 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 stored in accordance with the package insert are valid for testing, even if the expiration date on the collection tube has passed.

M. 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.

N. 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.

O. Discard used materials without passing over any other container.

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

Q. 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.

R. 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.

S. 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.

Assay Related

T. 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.

U. Use Universal Precautions when handling controls.

V. Avoid microbial and ribonuclease contamination of reagents.

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

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

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

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

Note: *Hazard Communication reflects the 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** ***HEPES 25 - 30%*** — 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** ***TRITON X-100 1 - 5%*** ***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.
—	**Probe Reagent** ***LAURYL SULFATE LITHIUM SALT 35 - 40%*** ***SUCCINIC ACID 10 - 15%*** — 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.
 	**Selection Reagent** ***BORIC ACID 0 - 10%*** ***TRITON X-100 0 - 10%*** ***SODIUM HYDROXIDE 0 - 10%*** **Danger** H315 - Causes skin irritation. H360FD - May damage fertility. May damage the unborn child. P264 - Wash face, hands and any exposed skin thoroughly after handling. P280 - Wear protective gloves/protective clothing/eye protection/face protection. P302 + P352 - IF ON SKIN: Wash with plenty of water and soap. P321 - Specific treatment (see supplemental first aid instructions on the SDS). P332 + P313 - If skin irritation occurs: Get medical advice/attention. P362 + P364 - Take off contaminated clothing and wash it before reuse. P201 - Obtain special instructions before use. P202 - Do not handle until all safety precautions have been read and understood. P308 + P313 - IF exposed or concerned: Get medical advice/attention. P405 - Store locked up. 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 reagents and controls:

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2ºC to 8ºC		
Enzyme Reagent	2ºC to 8ºC		
Probe Reagent	2ºC to 8ºC		
Target Capture Reagent B	2ºC to 8ºC		
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		Single Use Vial
Negative Control	2ºC to 8ºC		Single Use Vial

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

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

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

E. Discard any unused reconstituted reagents and wTCR 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 onboard the Panther system have 72 hours of onboard 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 and Reconstituted 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, clinician-collected and patient-collected vaginal swab specimens, female and male

urine specimens, and PreservCyt solution 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)

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. Assay specimens 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. Specimens collected in PreservCyt Solution
   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 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 (Panther System) Kit

250 tests (2 boxes and 1 Controls kit) (Cat. No. 303163)

100 tests (2 boxes and 1 Controls kit) (Cat. No. 303209)

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

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

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

Materials Required But Available Separately

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

	Cat. No.
Panther System	303095
Panther Fusion System	PRD-04172
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 Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Aptima Urine Specimen Collection Kit for Male and Female Urine Specimens	301040
Aptima Urine Specimen Transport Tubes for Male and Female Urine Specimens	105575
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.*

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 plastic 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.*

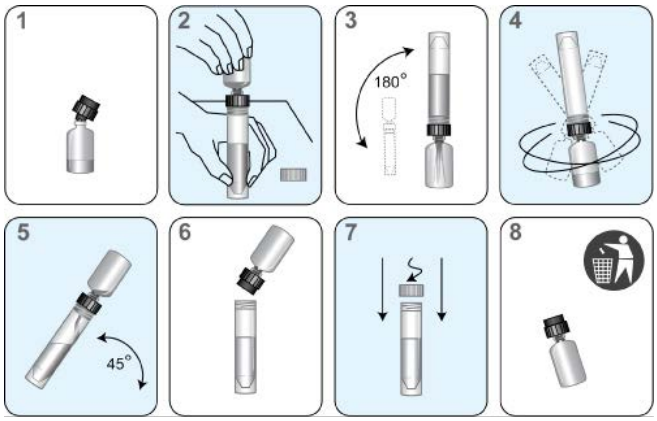

Figure 1. Reagent Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)
   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

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

   Option: *The reconstituted Amplification, Enzyme and Probe Reagents capped plastic bottles may be placed on a tube rocker set at a moderate speed and tilt until reagents reach room temperature and are thoroughly mixed.*

2. If reconstituted 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.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.
4. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

D. Specimen Handling

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

   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 for the Panther system, one pair of controls is required. The Positive Control for Trichomonas and Negative Control for Trichomonas 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.

If the results are positive, 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 for Trichomonas, which is labeled "NC CONTROL – TRICH," and the Positive Control for Trichomonas, 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 for Trichomonas 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.

Each laboratory should implement appropriate control procedures to satisfy local requirements. For assistance with out-of-range controls, contact Hologic Technical Support.

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 *Trichomonas 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 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, training of clinicians in proper specimen collection techniques is necessary. See *Specimen Collection and Storage* for instructions. For detailed information, refer to the appropriate instructions for use.

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. 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.

L. Performance of urine, vaginal swab, and PreservCyt solution Pap specimens has not been evaluated in adolescents less than 14 years of age.

M. The performance of gynecological specimens collected in the PreservCyt solution vial and processed with ThinPrep® Systems has not been evaluated with the Aptima TV assay.

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

O. 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.

P. Customers must independently validate an LIS transfer process.

Expected Values

Estimates of the positivity 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 on the Panther system is shown in Table 1 and Table 2 for the two multicenter clinical studies by clinical site and overall.

Table 1: Positivity of T. vaginalis as Determined by the Aptima Trichomonas vaginalis assay by Specimen Type and Collection Site

Specimen Type	% (# positive / # tested)									
	All Sites	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
FU	9.8 (64/650)	15.1 (8/53)	3.6 (2/55)	15.4 (2/13)	18.6 (8/43)	0.7 (1/136)	13.2 (10/76)	7.6 (11/144)	13.4 (11/82)	22.9 (11/48)
CVS	11.8 (80/678)	17.0 (9/53)	7.7 (4/52)	16.7 (2/12)	19.5 (8/41)	0.7 (1/145)	16.0 (12/75)	12.0 (21/175)	15.0 (12/80)	24.4 (11/45)
ES	11.2 (80/713)	20.4 (11/54)	8.9 (5/56)	12.5 (2/16)	17.1 (7/41)	0.6 (1/162)	20.2 (18/89)	9.1 (15/164)	13.3 (11/83)	20.8 (10/48)
PCyt	11.0 (81/739)	18.3 (11/60)	7.9 (5/63)	17.6 (3/17)	18.6 (8/43)	0.6 (1/167)	19.8 (17/86)	9.5 (16/169)	10.5 (9/86)	22.9 (11/48)

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

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

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 and Table 4 for two multicenter clinical studies. These calculations are based on the overall estimated sensitivity and specificity for each specimen type (See Table 5 and Table 6.).

Table 3: Hypothetical PPV and NPV of the Aptima Trichomonas vaginalis assay by Specimen Type

Specimen Type	Prevalence (%)	PPV (%)	NPV (%)
FU	1	52.2	99.9
	2	68.8	99.9
	5	85.0	99.7
	10	92.3	99.3
	15	95.0	98.9
	20	96.4	98.4
	25	97.3	97.9
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
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	52.4	100
	2	69.0	100
	5	85.2	100
	10	92.4	100
	15	95.1	100
	20	96.5	100
	25	97.3	100

PPV = positive predictive value; **NPV** = negative predictive value; **FU** = female urine; **CVS** = clinician-collected vaginal swab; **ES** = endocervical swab; **PCyt** = PreservCyt Solution Pap.

The PPV and NPV are derived for different hypothetical prevalence rates using the sensitivity and specificity estimates from the clinical performance study.

Table 4: Hypothetical PPV and NPV of the Aptima Trichomonas vaginalis assay by Specimen Type

Specimen Type	Prevalence (%)	PPV (%)	NPV (%)
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
FU	1	100	100
	2	100	100
	5	100	100
	10	100	100
	15	100	100
	20	100	100
	25	100	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

PPV = positive predictive value; **NPV** = negative predictive value; **PVS** = patient-collected vaginal swab; **FU** = female urine; **MU** = male urine.

The PPV and NPV are derived for different hypothetical prevalence rates using the sensitivity and specificity estimates from the clinical performance study.

Panther System Clinical Performance

Clinical Study

Two clinical studies were performed. Aptima TV assay clinical performance was estimated with clinician-collected vaginal swab, endocervical swab, female urine and PreservCyt solution 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 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. One first-catch urine, 3 vaginal swab, 1 endocervical swab, and 1 PreservCyt solution Pap specimens were collected from each subject. All specimens were clinician-collected except urine specimens.

PreservCyt solution 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.

A total of 651 urine, 689 vaginal swab, 737 endocervical swab, and 740 PreservCyt solution Pap specimens were tested with the Aptima TV assay on the Panther system. Specimens with initial invalid results were retested. One (1) urine, 11 vaginal swab, 24 endocervical swab, and 1 PreservCyt solution Pap specimens had final invalid results due to hardware or software errors; these specimens were excluded from the analyses.

The sensitivity of the Aptima TV assay using urine specimens on the Panther system and compared to a patient-infected status (PIS) that was determined using vaginal swab specimens was shown to be slightly lower than the sensitivity of other sample types. While this is not unexpected considering vaginal swabs are the preferred sample type for detection of trichomoniasis in women (12), the study design also had several limitations. As previously noted, the clinical performance of the Aptima TV assay on the Panther system was evaluated using remnant specimens collected from consenting subjects during a previous, prospective, multicenter clinical study of the Aptima TV assay on the Tigris DTS system, an automated system that predated the Panther system. Samples were stored frozen long-term before Panther testing (up to 18 months at –70°C) and a large number of samples had to be excluded from re-

testing, largely due to a lack of patient consent for additional testing after completion of the initial study on the Tigris DTS system.

Only 15 positive urine samples from asymptomatic patients were available for re-testing during the Panther study. Thus, a single sample that had previously tested positive during the initial Tigris DTS study but negative after long-term storage had a noticeable impact on the reported sensitivity of the assay for asymptomatic urine samples in the Panther study. The sensitivity and specificity of the Aptima TV assay using the Tigris DTS system as initially determined during the prospective clinical study likely better reflects the true sensitivity of the assay using urine specimens given the increased number of patient samples available for testing, the use of prospectively gathered specimens rather than those stored long-term before testing, and the determined equivalence between systems.

A total of 738 urine, 877 vaginal swab, 922 endocervical swab, and 813 PreservCyt solution Pap specimens were tested with the Aptima TV assay on the Tigris DTS system. In both the Tigris DTS study and the Panther study, the sensitivity for vaginal swabs, endocervical swabs, and samples collected in PreservCyt solution was 100% for both asymptomatic and symptomatic patients, but the performance of the assay using urine specimens was more variable.

A comparability study of the assay on the Tigris DTS system versus the Panther system showed high agreement between the two systems for all sample types indicated for use (> 95% positive and negative agreement). Overall agreement for all specimen types was 99.2% (95% CI 98.7–99.5) for the 2,056 specimens tested, and agreement among the 495 urine specimens tested was 99.6% (95% CI 98.5–99.9; positive agreement was 99.0% for all sample types and 96.2% for urine). An additional target capture reagent was added to the assay formulation prior to migration to the Panther system, and a separate comparability study showed that the additional reagent did not impact clinical performance using the Tigris DTS system. This study showed 99.5% (95% CI 98.7–99.8) overall agreement for all 758 samples tested and 100% (95% CI 98.1–100) overall agreement for 160 urine specimens tested by both versions of the assay (positive agreement was 100% for all sample types including urine). Given the high agreement between systems and assay versions, the clinical performance of the assay using urine specimens as determined by initial testing on the Tigris DTS system and with a larger sample size is therefore shown in Table 5.

Additionally, two studies in the scientific literature comparing the Aptima TV assay to two nucleic acid amplification tests that are FDA-cleared for urine specimens showed highly comparable performance with Aptima TV (13, 14). One of these reports showed 100% positive and negative agreement of the Aptima TV assay and the comparator test using 412 urine specimens (13). The other report describes testing of 1,793 female urine specimens during a multicenter clinical study and showed 99.4% positive agreement (95% CI 96.9–100, n=178/179) and 99.6% negative agreement (95% CI 99.1–99.8, n=1,607/1,614) between the Aptima TV assay and the comparator nucleic acid test (14). A third literature report compared Aptima TV testing of paired endocervical swab and urine specimens from 369 Canadian women, and found 99.2% concordance between sample types (15). Thus, it can be concluded that the Aptima TV assay performs as well as other commercially available tests and similarly to other sample types in detecting *T. vaginalis* from urine specimens, and the reported sensitivity of the assay determined using urine specimens on the Panther system is likely underestimated due to limitations of the study design.

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, 5833 (98.5%) had final valid results, and 89 (1.5%) had final invalid results 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 algorithm (CCA) interpretation as follows:

- Male urine CCA was derived from male urine specimens.
- Female urine CCA was derived from female urine specimens.
- Vaginal swab CCA was derived from patient-collected vaginal swab specimens.

Specimens were categorized as infected if a positive result occurred in at least two of the reference NAATs, and as not infected if at least 2 of the reference results were negative; the third (tie-breaker) reference was only required if the first 2 reference results were discordant. Specimens that could not be categorized as infected or not infected were excluded from the performance analyses. Performance of the Aptima TV assay was estimated relative to the specimen-specific CCA interpretation.

A total of 5502 specimens from 3820 evaluable subjects were included in the analyses comparing Aptima TV assay results to the specimen-specific CCA interpretation: 1785 patient-collected vaginal swab, 1782 female urine, 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 5, Table 6, and Table 7, including data from the two clinical studies. The infected status algorithm differed among the two studies. Table 5 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 Pap specimens.

Table 6 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 Pap specimens by cervical collection device. For PreservCyt solution Pap specimens, performance was similar across collection devices.

Table 7 shows the positive (PPA) and negative (NPA) percent agreement of the assay in female patient-collected vaginal swab, and female and male urine specimens. Prevalence was higher in symptomatic subjects.

Table 5: Performance Characteristics of the Aptima Trichomonas vaginalis Assay by Symptom Status

Specimen Type	Symptom Status	n	TP	FP[1]	TN	FN[2]	Prev %	Sensitivity % (95% CI)[3]	Specificity % (95% CI)[3]	PPV % (95% CI)[4]	NPV % (95% CI)[4]
CVS (Panther system)	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 (Panther system)	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 (Panther system)	Asymptomatic	324	18	1[g]	305	0	5.6	100 (82.4–100)	99.7 (98.2–99.9)	94.7 (76.5–99.9)	100 (98.9–100)
	Symptomatic	406	57	5[h]	344	0	14.0	100 (93.7–100)	98.6 (96.7–99.4)	91.9 (83.1–97.2)	100 (99.0–100)
	All	730	75	6[i]	649	0	10.3	100 (95.1–100)	99.1 (98.0–99.6)	92.6 (85.2–97.1)	100 (99.5–100)
Urine (Panther system)	Asymptomatic	279	13	1[j]	263	2[m]	5.4	86.7 (62.1–96.3)	99.6 (97.9–99.9)	92.9 (71.6–99.8)	99.2 (97.8–99.9)
	Symptomatic	361	46	4[k]	309	2[n]	13.3	95.8 (86.0–98.8)	98.7 (96.8–99.5)	92.0 (82.4–97.5)	99.4 (97.9–99.9)
	All	640	59	5[l]	572	4[o]	9.8	93.7 (84.8–97.5)	99.1 (98.0–99.6)	92.2 (84.0–97.1)	99.3 (98.3–99.8)
Urine (Tigris)	Asymptomatic	324	21	3	299	1	6.8	95.5 (78.2–99.2)	99.0 (97.1–99.7)	87.5 (71.4–96.9)	99.7 (98.4–100)
	Symptomatic	411	59	4	345	3	15.1	95.2 (86.7–98.3)	98.9 (97.1–99.6)	93.7 (85.7–98.1)	99.1 (97.7–99.8)
	All	735	80	7	644	4	11.4	95.2 (88.4–98.1)	98.9 (97.8–99.5)	92.0 (85.1–96.4)	99.4 (98.5–99.8)

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

[1] *T. vaginalis* NAAT results from a previous study (# positive results / # samples tested): [a]4/7; [b]3/4; [c]7/11; [d]1/5; [e]2/7; [f]3/12; [g]0/1; [h]3/5; [i]3/6; [j]1/1; [k]4/4; [l]5/5.

[2] *T. vaginalis* NAAT results from a previous study (# negative results / # samples tested): [m]1/2; [n]2/2; and [o]3/4.

[3] Score confidence interval.

[4] 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 6: Performance Characteristics of the Aptima Trichomonas vaginalis Assay in PreservCyt Solution Pap Specimens by Collection Device Type

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	391	48	3	340	0	12.3	100 (92.6–100)	99.1 (97.5–99.7)	94.1 (84.7–98.7)	100 (99.0–100)
Spatula/Cytobrush	339	27	3	309	0	8.0	100 (87.5–100)	99.0 (97.2–99.7)	90.0 (75.7–97.8)	100 (98.9–100)

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 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 7: Performance Characteristics of the Aptima TV Assay Female Patient-collected Vaginal Swab, and Male and Female Urine Specimens by Symptom Status

Specimen Type	Symptom Status[1]	n	TP	FP[2]	TN	FN[3]	Prev %	PPA % (95% CI)[4]	NPA % (95% CI)[4]
PVS	Asymptomatic	932	59	3[a]	868	2[a]	6.5	96.7 (88.8–99.1)	99.7 (99.0–99.9)
	Symptomatic	853	99	6[a]	748	0	11.6	100 (96.3–100)	99.2 (98.3–99.6)
	All	1785	158	9	1616	2	9.0	98.8 (95.6–99.7)	99.4 (99.0–99.7)
FU	Asymptomatic	949	64	0	885	0	6.7	100 (94.3–100)	100 (99.6–100)
	Symptomatic	833	94	0	739	0	11.3	100 (96.1–100)	100 (99.5–100)
	All	1782	158	0	1624	0	8.9	100 (97.6–100)	100 (99.8–100)
MU	Asymptomatic	1125	21	1[b]	1103	0	1.9	100 (84.5–100)	99.9 (99.5–100)
	Symptomatic	810	21	2[c]	787	0	2.6	100 (84.5–100)	99.7 (99.1–99.9)
	All	1935	42	3	1890	0	2.2	100 (91.6–100)	99.8 (99.5–99.9)

CI = confidence interval; **FN** = false negative; **FP** = false positive; **FU** = female urine; **MU** = male urine; **NPA** = negative percent agreement; **PPA** = positive percent agreement; **Prev** = prevalence; **TN** = true negative; **TP** = true positive.

[1]Patient-collected vaginal swab, female urine and male urine sample 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.

RLU Distribution of Aptima Trichomonas vaginalis Controls

The distribution of the RLU values for the Aptima TV assay controls is presented in Table 8 for all valid Aptima TV assay runs performed during Clinical Study 1 and Clinical Study 2.

Table 8: 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
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 calculated; **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.

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 0.008 TV/mL in PreservCyt solution Pap specimen matrix, panels containing 0.003 TV/mL in urine, and panels containing 0.001 TV/mL in swab specimen matrix.

Cross-Reactivity in the Presence of Microorganisms

Specificity

Specificity 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 9. No cross-reactivity or significant effect on Aptima TV assay specificity was observed with any of the organisms tested.

Sensitivity

Sensitivity of the Aptima TV assay was evaluated by testing the same organisms (Table 9) in STM spiked with *T. vaginalis* lysate to a final concentration of 2.5 TV/mL (25 replicates of each isolate). *T. vaginalis* lysate was also spiked into STM, urine, and PreservCyt solution in STM to a final concentration of 0.01 TV/mL (25 replicates of each isolate). Sensitivity of the Aptima TV assay was not significantly affected by the presence of the microorganisms tested, except in the presence of *Trichomonas tenax* and *Pentatrichomonas hominis* (where lower signal outputs were observed). *T. tenax* is a commensal of the oral cavity and *Pentatrichomonas hominis* is a commensal of the large intestine.

At the assay limit of detection (0.01 TV/mL), a slight inhibitory effect was observed on expected RLU values by *Dientamoeba fragilis,* but assay sensitivity was not impacted and *D. fragilis* is found in the gastrointestinal tract.

Table 9: Microorganisms Tested in the Aptima Trichomonas vaginalis assay

Microorganism	Concentration	Microorganism	Concentration
Acinetobacter lwoffii	$1x10^6$ 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
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

Interference

The following substances were individually spiked into STM 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).

The effects of urine metabolites were tested by the addition of KOVA-Trol I High Abnormal w/ Urobilinogen Urinalysis Control diluted into Urine Transport Medium (UTM) in place of urine. This human urine-based urinalysis control material contains potential interferents such as protein (albumin), bilirubin, glucose, ketones, red blood cells, nitrite, urobilinogen and leukocytes. Glacial acetic acid was tested by spiking into PreservCyt solution-STM (10% final concentration).

No interference was observed with any of the tested substances in the Aptima TV assay with the exception of porcine gastric mucus, which exhibited lower signal output when present at a final concentration of 1% (vol/vol or wt/vol).

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 or negative PreservCyt solution Pap specimens with specimen transport medium. The positive panel members were created by spiking the urine matrix or PreservCyt solution 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 10 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 10: 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 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.

Carryover

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 the vaginal swab, urine, and PreservCyt solution Pap specimens were generated with negative clinical specimens spiked with *T. vaginalis* to a final concentration of 250 TV/mL. Greater than 97% positivity was observed in all matrices (vaginal swab, urine, and PreservCyt solution Pap) at all times and temperatures tested confirming the validity of the maximum storage times and temperatures described in *Specimen Collection and Storage*.

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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-31091-001 Rev. 003
2024-11

Revision History	Date	Description
AW-31091 Rev. 001	April 2024	• Creation of a commercial version of Aptima Trichomonas vaginalis assay IFU, AW-31091 Rev. 001, for IVDR compliance (ExUS) based on a regulatory submission version of Aptima Trichomonas vaginalis assay IFU, AW-31091 Rev. 002 (ExUS).
AW-31091 Rev. 002	June 2024	• Updated the SDS section with any newer information. • Implemented administrative updates throughout.
AW-31091 Rev. 003	November 2024	• Updated SDS section with new information. • Added routine administrative edits • Change trademark to register trademarks