Aptima® Trichomonas vaginalis Assay

For *in vitro* diagnostic use.

For US export only.

Tigris® DTS®

Panther®

General Information

Intended Use

The Aptima Trichomonas vaginalis 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 Tigris DTS System or the Panther System.

The assay may be used to test the following specimens from symptomatic or asymptomatic women: clinician-collected endocervical swabs, clinician-collected vaginal swabs, female urine specimens, and specimens collected in PreservCyt® Solution.

Summary and Explanation of the Test

Trichomonas 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 complain 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 (3, 4, 5).

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 (6, 7).

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 (7). 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.

The Aptima Trichomonas vaginalis Assay is a nucleic acid test that utilizes Target Capture, Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (HPA) technologies.

Principles of the Procedure

The Aptima Trichomonas vaginalis 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 Trichomonas vaginalis Assay is performed in the laboratory, the target rRNA is isolated from the specimens by the use of 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 hybridization (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 additional specific warnings and precautions, refer to the *Tigris DTS System Operator's Manual*.

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

Laboratory Related

D. Use only supplied or specified disposable laboratory ware.

E. 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.

F. **Warning: Irritants and Corrosives.** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash with water. If these fluids spill, dilute the spill with water before wiping dry.

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

Specimen Related

H. Expiration dates for the specimen transfer kits pertain to the collection/transfer of specimens and not to specimen testing. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they have been transported and stored in accordance with the package insert, even if the expiration date on the transfer tube has passed.

I. 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.

J. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over any container. Change gloves if they come in contact with specimen.

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

L. 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.

M. 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.

N. 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

O. Store reagents at the specified temperatures. Performance of the assay may be affected by use of improperly stored reagents.

P. Use Universal Precautions when handling controls.

Q. Avoid microbial and ribonuclease contamination of reagents.

R. Do not use kit after its expiration date.

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

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2ºC to 8ºC:

Aptima *Trichomonas vaginalis* Amplification Reagent

Aptima *Trichomonas vaginalis* Enzyme Reagent

Aptima *Trichomonas vaginalis* Probe Reagent

Aptima *Trichomonas vaginalis* Assay Target Capture Reagent B

Aptima *Trichomonas vaginalis* Controls

B. The following reagents are stable when stored at room temperature (15°C to 30°C):

Aptima *Trichomonas vaginalis* Amplification Reconstitution Solution

Aptima *Trichomonas vaginalis* Enzyme Reconstitution Solution

Aptima *Trichomonas vaginalis* Probe Reconstitution Solution

Aptima *Trichomonas vaginalis* Target Capture Reagent

Aptima *Trichomonas vaginalis* Selection Reagent

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

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

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 from 250-test bottles stored on-board the Tigris DTS System have 48 hours of on-board stability.

H. Reagents from 100-test bottles stored on-board the Tigris DTS System have 96 hours of on-board stability.

I. Reagents stored on-board the Panther System have 72 hours of on-board stability.

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

K. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light.

L. **Do not freeze reagents**.

Specimen Collection and Storage

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

- Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- Aptima Urine Collection Kit for Male and Female Urine Specimens
- Aptima Vaginal Swab Specimen Collection Kit
- Aptima Multitest Swab Specimen Collection Kit
- Aptima Specimen Transfer Kit (for use with gynecological samples collected in PreservCyt Solution)

A. Instructions for collection

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

B. Specimen transport and storage before testing:

1. 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 12 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 12 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 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 12 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 RCF (Relative Centrifugal Force) 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.*

Tigris DTS System

Reagents for the Aptima Trichomonas vaginalis Assay are listed below for the Tigris DTS System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Aptima Trichomonas vaginalis Assay Kit

250 tests (2 boxes and 1 Controls kit) (Cat. No. 303164)

100 test (2 boxes and 1 Controls kit) (Cat. No. 303174)

Aptima Trichomonas vaginalis 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	**Aptima *Trichomonas vaginalis* Amplification Reagent** *Primers and nucleotides dried in buffered solution containing < 5% bulking agent.*	1 vial	1 vial
E	**Aptima *Trichomonas vaginalis* Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial	1 vial
P	**Aptima *Trichomonas vaginalis* Probe Reagent** *Chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial	1 vial
TCR-B	**Aptima *Trichomonas vaginalis* Assay Target Capture Reagent B** *Buffered solution containing < 5% detergent.*	1 x 0.56 mL	1 x 0.30 mL

Aptima Trichomonas vaginalis 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	**Aptima *Trichomonas vaginalis* Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL	1 x 11.9 mL
ER	**Aptima *Trichomonas vaginalis* Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL	1 x 6.3 mL

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

PR	**Aptima *Trichomonas vaginalis* Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL	1 x 15.2 mL
S	**Aptima *Trichomonas vaginalis* Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL	1 x 43.0 mL
TCR	**Aptima *Trichomonas vaginalis* 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	**Aptima *Trichomonas vaginalis* Negative Control** *Non-infectious non-target nucleic acid in a buffered solution containing < 5% detergent.*	5 x 1.7 mL
PC	**Aptima *Trichomonas vaginalis* 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 specified otherwise.*

		Cat. No.
Tigris DTS System		105118
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*		302382
Aptima Auto Detect Kit		301048
Aptima System Fluid Preservative Kit		302380
Tips, 1000 µL conductive, liquid sensing		10612513 (Tecan)
Tigris DTS System Run Kit		301191
Multi-tube Units (MTUs)	*104772-02*	
MTU/Tiplet Waste Bag Kit	*900907*	
MTU Waste Deflectors	*900931*	
MTU Waste Covers	*105523*	
Aptima Specimen Transfer Kit *for use with specimens in PreservCyt Solution*		301154C
Aptima Vaginal Swab Specimen Collection Kit		301162
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 7% (0.7 M to 1.0 M) sodium hypochlorite solution		—
Water for the Tigris DTS System *consult the Tigris DTS System Operator's Manual for specifications*		—
Disposable gloves		—
SysCheck calibration standard		301078
Aptima penetrable caps		105668
Replacement non-penetrable caps		103036A
Replacement Caps for 250 test kits		—
Amplification and Probe reagent reconstitution solutions		
	CL0041 (100 caps)	
Enzyme Reagent reconstitution solution	*501616 (100 caps)*	
TCR and Selection Reagent solutions	*CL0040 (100 caps)*	

	Cat. No.
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

Tigris DTS System Test Procedure

Note: *See the Tigris DTS System Operator's Manual for additional Tigris DTS 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% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a 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 Tigris DTS 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 lyophilized 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 vial and firmly insert the notched end of the reconstitution collar into the 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 bottle opening (Figure 1, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

g. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (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 plastic bottle.

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

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

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

Warning: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Tigris DTS System.*

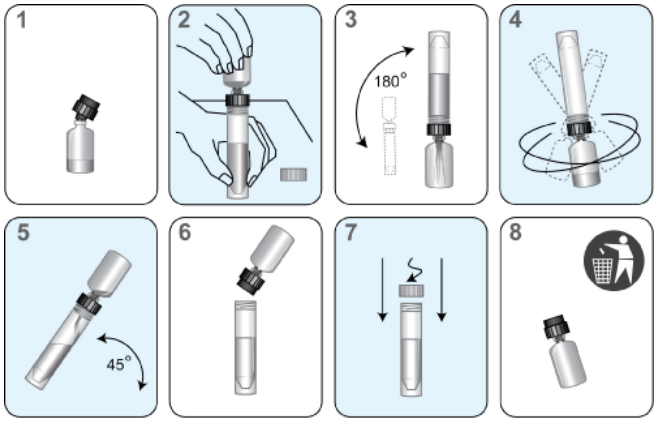

Figure 1. Tigris DTS System or Panther System Reconstitution Process

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 reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   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.
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 60°C for 1 to 2 minutes. After this heat step, Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion.
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 Tigris DTS System will recognize and reject bottles that have been topped off.

D. Sample 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 blue Aptima collection swab in a unisex swab specimen transport tube.
   b. The presence of a single pink Aptima collection swab in a multitest or vaginal 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 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 3 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 3 aliquots from the specimen tube can lead to insufficient volume errors.*

E. System Preparation

Set up the system and worklist according to the instructions in the *Tigris DTS System Operator's Manual* and the *Procedural Notes*.

Procedural Notes

A. Controls

1. To work properly with the Aptima Trichomonas vaginalis Assay software, controls are required at the front and end of a worklist. The Aptima Negative Control for Trichomonas must be in the first position and the second to last tube position of the last rack of the worklist. The Aptima Positive Control for Trichomonas must be in the second position and the last tube position of the last rack of the worklist.
2. Each control tube can be tested once. Attempts to pipette more than once from the tube can lead to insufficient volume errors.

B. Temperature

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

C. Gloves

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 Tigris DTS 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 swab transport medium, and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

If the results are positive, see *Test Interpretation - QC/Patient Results*. For additional Tigris DTS System-specific contamination monitoring information, see the *Tigris DTS System Operator's Manual*.

Panther System

Reagents for the Aptima Trichomonas vaginalis Assay are listed below for the Panther System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Aptima Trichomonas vaginalis Assay Kit

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

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

Aptima Trichomonas vaginalis 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	**Aptima *Trichomonas vaginalis* Amplification Reagent** *Primers and nucleotides dried in buffered solution containing < 5% bulking agent.*	1 vial	1 vial
E	**Aptima *Trichomonas vaginalis* Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial	1 vial
P	**Aptima *Trichomonas vaginalis* Probe Reagent** *Chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial	1 vial
TCR-B	**Aptima *Trichomonas vaginalis* Assay Target Capture Reagent B** *Buffered solution containing < 5% detergent.*	1 x 0.56 mL	1 x 0.30 mL

Aptima Trichomonas vaginalis 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	**Aptima *Trichomonas vaginalis* Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL	1 x 11.9 mL
ER	**Aptima *Trichomonas vaginalis* Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL	1 x 6.3 mL

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

PR	**Aptima *Trichomonas vaginalis* Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL	1 x 15.2 mL
S	**Aptima *Trichomonas vaginalis* Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL	1 x 43.0 mL
TCR	**Aptima *Trichomonas vaginalis* 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	**Aptima *Trichomonas vaginalis* Negative Control** *Non-infectious non-target nucleic acid in a buffered solution containing < 5% detergent.*	5 x 1.7 mL
PC	**Aptima *Trichomonas vaginalis* 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
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 conductive, liquid sensing	10612513 (Tecan)
Aptima Specimen Transfer Kit *for use with specimens in PreservCyt Solution*	301154C
Aptima Vaginal Swab Specimen Collection Kit	301162
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 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable gloves	—
SysCheck calibration standard	301078
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

Panther System Test Procedure

Note: *See the Panther 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 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 vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 2, 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 bottle opening (Figure 2, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 2, Step 3).
   g. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (Figure 2, 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 2, Step 5). Allow all of the liquid to drain back into the plastic bottle.
   i. Remove the reconstitution collar and glass vial (Figure 2, Step 6).

j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 2, Step 7).

k. Discard the reconstitution collar and glass vial (Figure 2, Step 8).

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

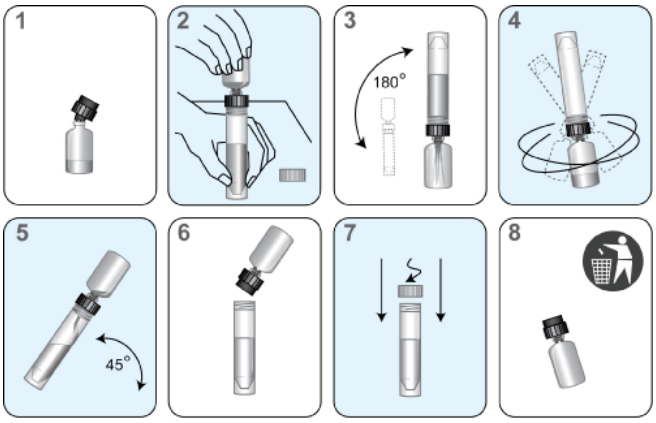

Figure 2. Tigris DTS System or Panther System Reconstitution Process

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 reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   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.
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 specimens.**
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 or vaginal 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 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 3 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 3 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 System Operator's Manual* and *Procedural Notes*.
2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Panther Aptima Assay software, one pair of controls is required. The Aptima Positive Control for Trichomonas and Aptima 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 swab transport medium, and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

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 Tigris DTS System or Panther System Aptima Trichomonas 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 Tigris DTS System or 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 Aptima Negative Control for Trichomonas, which is labeled "NC CONTROL – TRICH," and the Aptima 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 Aptima Positive Control for Trichomonas which is labeled "PC CONTROL + TRICH" contains non-infectious *T. vaginalis* rRNA.

The Aptima Trichomonas vaginalis 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 Tigris DTS System or 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 the requirements of CLIA regulations (section 493.1256).

Note: *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 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, 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 Trichomonas vaginalis Assay since nucleic acid may persist following appropriate antimicrobial therapy.

H. Results from the Aptima Trichomonas vaginalis 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 Trichomonas vaginalis 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. The Aptima Trichomonas vaginalis Assay has not been validated for use with vaginal swab specimens collected by patients.

M. Performance of the vaginal swab specimen has not been evaluated in pregnant women.

N. Performance of the vaginal swab and PreservCyt Solution liquid Pap specimen has not been evaluated in women less than 14 years of age.

O. The performance of the Tigris DTS System has not been determined at altitudes above 2240 m (7355 feet). Additional volumetric verifications and assay specific studies will be performed prior to, or as part of, the installation and acceptance process in laboratories above 2240 m (7355 feet) altitude.

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

Q. 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.

R. Customers must independently validate an LIS transfer process.

S. The performance of gynecological specimens collected in the PreservCyt Solution vial and processed with the ThinPrep® 2000 System has not been established for the Aptima Trichomonas vaginalis Assay.

Tigris DTS System Assay Performance

Prevalence

The prevalence of *T. vaginalis* in different populations depends on patient risk factors such as age, lifestyle, the presence or absence of symptoms, and the sensitivity of the test in detecting the infection. A summary of the prevalence of *T. vaginalis*, by specimen type, as determined by the Aptima Trichomonas vaginalis Assay in the clinical trial is shown in Table 1.

Table 1: Prevalence 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**
Urine	11.8 (87/735)	19.0 (11/58)	6.8 (5/73)	14.3 (2/14)	16.5 (16/97)	0.7 (1/136)	20.5 (18/88)	7.6 (8/105)	12.2 (12/98)	21.2 (14/66)
CVS	13.6 (119/875)	22.0 (13/59)	9.5 (7/74)	16.7 (2/12)	20.1 (28/139)	0.7 (1/146)	23.2 (22/95)	10.5 (20/191)	12.6 (12/95)	21.9 (14/64)
ES	12.9 (119/920)	19.4 (12/62)	9.5 (7/74)	17.6 (3/17)	21.1 (31/147)	0.6 (1/165)	22.4 (22/98)	9.8 (19/193)	11.3 (11/97)	19.4 (13/67)
PCyt	11.8 (96/813)	19.4 (12/62)	8.5 (6/71)	17.6 (3/17)	16.3 (17/104)	0.6 (1/167)	23.5 (23/98)	7.8 (10/129)	11.2 (11/98)	19.4 (13/67)

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

Clinical Performance

A pivotal, prospective, multicenter clinical trial was conducted to establish the performance characteristics for the Aptima Trichomonas vaginalis Assay. One thousand twenty-five (1,025) symptomatic and asymptomatic women were enrolled from nine US clinical sites, including obstetrics and gynecology, family planning, and STD clinics. Up to 6 specimens were collected from each subject (1 first-catch urine, 3 vaginal swab, 1 endocervical swab, and 1 PreservCyt Solution liquid Pap specimen). All specimens were clinician-collected except urine specimens. 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 4 specimens were prepared for Aptima Trichomonas vaginalis Assay testing in accordance with the appropriate Aptima specimen collection kit package insert instructions. Testing with the Aptima Trichomonas vaginalis Assay was conducted at three external laboratories in accordance with package insert instructions.

Performance characteristics of the Aptima Trichomonas vaginalis 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.

Of the evaluable specimens, a total of 738 urine, 877 vaginal swab, 922 endocervical swab, and 813 PreservCyt Solution liquid Pap specimens were tested with the Aptima Trichomonas vaginalis Assay. Specimens with initial invalid results were retested. Three (3) urine, two (2) vaginal swab, and two (2) endocervical swab specimens had final invalid results due to hardware errors or specimen issues; these specimens were excluded from the analyses.

Table 2 shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Aptima Trichomonas vaginalis Assay and the prevalence of *T. vaginalis* (based on the infected status) in each specimen type. Performance was similar across specimen types.

Table 2: Performance Characteristics of the Aptima Trichomonas vaginalis Assay

Specimen Type	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
Urine	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)
CVS	875	111	8	756	0	12.7	100 (96.7-100)	99.0 (97.9-99.5)	93.3 (87.6-97.0)	100 (99.5-100)
ES	920	114	5	801	0	12.4	100 (96.7-100)	99.4 (98.6-99.7)	95.8 (90.7-98.6)	100 (99.6-100)
PCyt	813	93	3	717	0	11.4	100 (96.0-100)	99.6 (98.8-99.9)	96.9 (91.4-99.3)	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.
[1]Score confidence interval.
[2]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 3 shows the sensitivity, specificity, PPV, and NPV of the Aptima Trichomonas vaginalis Assay and the prevalence of *T. vaginalis* (based on the infected status) in each specimen type by symptom status. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. For each specimen type, performance was similar in symptomatic and asymptomatic subjects. Prevalence was higher in symptomatic women.

Table 3: Performance Characteristics of the Aptima Trichomonas vaginalis Assay by Symptom Status

Specimen Type	Symptom Status	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
Urine	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)
CVS	Asymptomatic	345	24	4	317	0	7.0	100 (86.2-100)	98.8 (96.8-99.5)	85.7 (70.3-95.6)	100 (98.9-100)
	Symptomatic	530	87	4	439	0	16.4	100 (95.8-100)	99.1 (97.7-99.6)	95.6 (89.5-98.8)	100 (99.2-100)
ES	Asymptomatic	372	26	1	345	0	7.0	100 (87.1-100)	99.7 (98.4-99.9)	96.3 (82.4-99.9)	100 (99.0-100)
	Symptomatic	548	88	4	456	0	16.1	100 (95.8-100)	99.1 (97.8-99.7)	95.7 (89.6-98.8)	100 (99.2-100)
PCyt	Asymptomatic	353	23	0	330	0	6.5	100 (85.7-100)	100 (98.8-100)	100 (86.2-NC)	100 (99.0-100)
	Symptomatic	460	70	3	387	0	15.2	100 (94.8-100)	99.2 (97.8-99.7)	95.9 (88.9-99.1)	100 (99.1-100)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, NC = not calculable, PCyt = PreservCyt Solution liquid Pap, Prev = prevalence, TN = true negative, TP = true positive.
[1]Score confidence interval.
[2]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. Some confidence limits could not be calculated due to undefined results in the formulas.

Table 4 shows the sensitivity, specificity, PPV, and NPV of the Aptima Trichomonas vaginalis Assay and the prevalence of *T. vaginalis* (based on the infected status) in each specimen type by collection site. For each specimen type, performance was similar across collection sites. Prevalence varied across collection sites, as expected.

Table 4: Performance Characteristics of the Aptima Trichomonas vaginalis Assay by Collection Site

Site	Specimen Type	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
1	Urine	58	10	1	46	1	19.0	90.9 (62.3-98.4)	97.9 (88.9-99.6)	90.9 (66.5-99.7)	97.9 (91.2-99.9)
	CVS	59	12	1	46	0	20.3	100 (75.8-100)	97.9 (88.9-99.6)	92.3 (69.3-99.8)	100 (93.7-100)
	ES	62	12	0	50	0	19.4	100 (75.8-100)	100 (92.9-100)	100 (77.1-NC)	100 (94.0-100)
	PCyt	62	12	0	50	0	19.4	100 (75.8-100)	100 (92.9-100)	100 (77.1-NC)	100 (94.0-100)
2	Urine	73	5	0	67	1	8.2	83.3 (43.6-97.0)	100 (94.6-100)	100 (60.0-NC)	98.5 (94.6-100)
	CVS	74	6	1	67	0	8.1	100 (61.0-100)	98.5 (92.1-99.7)	85.7 (52.7-99.6)	100 (96.1-100)
	ES	74	6	1	67	0	8.1	100 (61.0-100)	98.5 (92.1-99.7)	85.7 (52.7-99.6)	100 (96.1-100)
	PCyt	71	6	0	65	0	8.5	100 (61.0-100)	100 (94.4-100)	100 (62.6-NC)	100 (95.9-100)
3	Urine	14	1	1	12	0	7.1	100 (20.7-100)	92.3 (66.7-98.6)	50.0 (3.0-97.5)	100 (92.1-100)
	CVS	12	2	0	10	0	16.7	100 (34.2-100)	100 (72.2-100)	100 (32.1-NC)	100 (85.6-100)
	ES	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)
	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	Urine	97	15	1	80	1	16.5	93.8 (71.7-98.9)	98.8 (93.3-99.8)	93.8 (74.4-99.8)	98.8 (94.4-100)
	CVS	139	27	1	111	0	19.4	100 (87.5-100)	99.1 (95.1-99.8)	96.4 (83.2-99.9)	100 (97.0-100)
	ES	147	30	1	116	0	20.4	100 (88.6-100)	99.1 (95.3-99.8)	96.8 (84.6-99.9)	100 (97.1-100)
	PCyt	104	17	0	87	0	16.3	100 (81.6-100)	100 (95.8-100)	100 (82.5-NC)	100 (96.3-100)
5	Urine	136	1	0	135	0	0.7	100 (20.7-100)	100 (97.2-100)	100 (6.4-NC)	100 (99.3-100)
	CVS	146	1	0	145	0	0.7	100 (20.7-100)	100 (97.4-100)	100 (6.4-NC)	100 (99.3-100)
	ES	165	1	0	164	0	0.6	100 (20.7-100)	100 (97.7-100)	100 (6.4-NC)	100 (99.4-100)
	PCyt	167	1	0	166	0	0.6	100 (20.7-100)	100 (97.7-100)	100 (6.4-NC)	100 (99.4-100)
6	Urine	88	17	1	69	1	20.5	94.4 (74.2-99.0)	98.6 (92.3-99.7)	94.4 (76.7-99.8)	98.6 (93.4-100)
	CVS	95	21	1	73	0	22.1	100 (84.5-100)	98.6 (92.7-99.8)	95.5 (79.5-99.9)	100 (95.6-100)
	ES	98	21	1	76	0	21.4	100 (84.5-100)	98.7 (93.0-99.8)	95.5 (79.5-99.9)	100 (95.8-100)
	PCyt	98	22	1	75	0	22.4	100 (85.1-100)	98.7 (92.9-99.8)	95.7 (80.3-99.9)	100 (95.7-100)
7	Urine	105	7	1	97	0	6.7	100 (64.6-100)	99.0 (94.4-99.8)	87.5 (56.3-99.6)	100 (97.2-100)
	CVS	191	18	2	171	0	9.4	100 (82.4-100)	98.8 (95.9-99.7)	90.0 (71.7-98.7)	100 (98.1-100)
	ES	193	18	1	174	0	9.3	100 (82.4-100)	99.4 (96.8-99.9)	94.7 (76.6-99.9)	100 (98.1-100)
	PCyt	129	9	1	119	0	7.0	100 (70.1-100)	99.2 (95.4-99.9)	90.0 (62.2-99.7)	100 (97.5-100)
8	Urine	98	11	1	86	0	11.2	100 (74.1-100)	98.9 (93.8-99.8)	91.7 (67.0-99.8)	100 (96.5-100)
	CVS	95	11	1	83	0	11.6	100 (74.1-100)	98.8 (93.6-99.8)	91.7 (67.0-99.8)	100 (96.4-100)
	ES	97	11	0	86	0	11.3	100 (74.1-100)	100 (95.7-100)	100 (75.3-NC)	100 (96.5-100)
	PCyt	98	11	0	87	0	11.2	100 (74.1-100)	100 (95.8-100)	100 (75.3-NC)	100 (96.5-100)
9	Urine	66	13	1	52	0	19.7	100 (77.2-100)	98.1 (90.1-99.7)	92.9 (70.9-99.8)	100 (94.3-100)
	CVS	64	13	1	50	0	20.3	100 (77.2-100)	98.0 (89.7-99.7)	92.9 (70.9-99.8)	100 (94.1-100)
	ES	67	13	0	54	0	19.4	100 (77.2-100)	100 (93.4-100)	100 (78.5-NC)	100 (94.4-100)
	PCyt	67	13	0	54	0	19.4	100 (77.2-100)	100 (93.4-100)	100 (78.5-NC)	100 (94.4-100)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, NC = not calculable, PCyt = PreservCyt Solution liquid Pap, Prev = prevalence, TN = true negative, TP = true positive.

[1]Score confidence interval.

[2]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. Some confidence limits could not be calculated due to undefined results in the formulas.

Table 5 shows the sensitivity, specificity, PPV, and NPV of the Aptima Trichomonas vaginalis Assay 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 5: Performance Characteristics of the Aptima Trichomonas vaginalis Assay in PreservCyt Solution Liquid Pap Specimens by Collection Device Type

Collection Device	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
Broom-type Device	447	62	1	384	0	13.9	100 (94.2-100)	99.7 (98.5-100)	98.4 (91.8-100)	100 (99.1-100)
Spatula/Cytobrush	366	31	2	333	0	8.5	100 (89.0-100)	99.4 (97.8-99.8)	93.9 (81.2-99.2)	100 (99.0-100)

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

[1]Score confidence interval.

[2]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.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated PPV and NPV of the Aptima Trichomonas vaginalis Assay across different hypothetical prevalence rates are shown for each specimen type in Table 6. These calculations are based on the overall estimated sensitivity and specificity for each specimen type.

Table 6: Hypothetical PPV and NPV of the Aptima Trichomonas vaginalis Assay by Specimen Type

Specimen Type	Prevalence (%)	PPV (%)	NPV (%)
Urine	1	47.2	100
	2	64.4	99.9
	5	82.3	99.7
	10	90.8	99.5
	12	92.4	99.3
	15	94.0	99.2
	20	95.7	98.8
	25	96.7	98.4
CVS	1	49.1	100
	2	66.1	100
	5	83.4	100
	10	91.4	100
	12	92.9	100
	15	94.4	100
	20	96.0	100
	25	97.0	100
ES	1	62.0	100
	2	76.7	100
	5	89.5	100
	10	94.7	100
	12	95.6	100
	15	96.6	100
	20	97.6	100
	25	98.2	100
PCyt	1	70.8	100
	2	83.0	100
	5	92.7	100
	10	96.4	100
	12	97.0	100
	15	97.7	100
	20	98.4	100
	25	98.8	100

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

The PPV and NPV are derived for different hypothetical prevalence rates using the sensitivity and specificity estimates from the clinical performance study. Sensitivity was 95.2% in urine specimens and 100% in vaginal swab, endocervical swab, and PreservCyt Solution liquid Pap specimens. Specificity was 98.9% in urine specimens, 99.0% in vaginal swab specimens, 99.4% in endocervical swab specimens, and 99.6% in PreservCyt Solution liquid Pap specimens.

RLU Distribution of Aptima Trichomonas vaginalis Controls

The distribution of the RLU values for the Aptima *Trichomonas vaginalis* Negative Control and the Aptima *Trichomonas vaginalis* Positive Control from all valid Aptima Trichomonas vaginalis Assay worklists performed during the clinical performance study is presented in Table 7.

Table 7: RLU Distribution of Aptima Trichomonas vaginalis Negative and Positive Controls

Control	Statistics	Total RLU (×1000)
Negative	N	58
	Mean	2.5
	SD	1.93
	Median	2.0
	Minimum	1
	Maximum	10
	CV%	78.3
Positive	N	58
	Mean	1206.3
	SD	91.37
	Median	1191.5
	Minimum	986
	Maximum	1381
	CV%	7.6

RLU = relative light unit.

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.

Assay Reproducibility

Aptima Trichomonas vaginalis Assay reproducibility was evaluated at three external US laboratories using the Tigris DTS System. Testing was performed over six days using three lots of assay reagents and six operators (two at each site). Reproducibility panels were created by spiking either urine matrix or PreservCyt Solution liquid Pap matrix with the appropriate amount of *T. vaginalis* lysate. Final *T. vaginalis* concentrations ranged from 0 to 1 TV/mL.

Table 8 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 worklists, within worklists, and overall (Total). Percent agreement with expected results is also shown. Samples with valid results were included in the analyses.

Table 8: Aptima Trichomonas vaginalis Assay Reproducibility Study

				Between Sites		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
Conc	N	Agmt (%)	Mean RLU	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
PreservCyt Solution Liquid Pap Matrix Samples															
Neg	106	100.0	2.0	1.1	56.8	0.0	0.0	0.0	0.0	0.4	21.3	0.8	42.5	1.5	74.1
HNeg	106	92.5	58.3	17.2	29.4	0.0	0.0	11.1	19.1	0.0	0.0	22.2	38.0	30.2	51.7
MPos	108	98.1	367.0	32.8	8.9	0.0	0.0	57.5	15.7	51.0	13.9	140.6	38.3	163.6	44.6
HPos	107	100.0	1110.4	53.9	4.9	0.0	0.0	109.6	9.9	60.9	5.5	77.1	6.9	156.8	14.1
Urine Matrix Samples															
Neg	108	100.0	2.1	1.0	45.7	0.0	0.0	0.0	0.0	0.0	0.0	1.3	62.4	1.7	77.3
HNeg	107	97.2	60.2	11.2	18.7	0.0	0.0	9.6	15.9	9.8	16.2	12.0	19.9	21.4	35.6
MPos	107	100.0	781.6	53.2	6.8	0.0	0.0	66.6	8.5	56.0	7.2	83.7	10.7	131.9	16.9
HPos	108	98.1	1122.8	49.5	4.4	15.0	1.3	119.3	10.6	109.2	9.7	106.9	9.5	200.7	17.9

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 was numerically negative. This occurred if the variability due to those factors was very small. In these cases, SD and CV are shown as 0.

Analytical Sensitivity

Sensitivity panel members containing 0.1 TV/mL in urine specimen matrix, PreservCyt liquid Pap specimen matrix, and vaginal swab matrix (90 replicates per matrix) were prepared with two strains of *T. vaginalis* (one Metronidazole-susceptible strain and one Metronidazole-resistant strain). Testing showed 100% positivity in all specimen matrices and in both *T. vaginalis* strains.

Cross-Reactivity in the Presence of Microorganisms

Specificity

Specificity of the Aptima Trichomonas vaginalis Assay was evaluated by testing various microorganisms, including common flora of the genitourinary tract, opportunistic organisms, and closely related organisms. Testing was conducted in specimen transport medium (STM), PreservCyt liquid Pap, and urine matrices with 25 replicates of each isolate per matrix. The list of organisms and the concentrations tested are provided in Table 9. No cross-reactivity or significant effect on Aptima Trichomonas vaginalis Assay specificity was observed with any of the organisms tested.

Sensitivity

Sensitivity of the Aptima Trichomonas vaginalis Assay was evaluated by testing the same organisms (Table 9) in STM, PreservCyt liquid Pap, and urine matrices spiked with *T. vaginalis* lysate to a final concentration of 2.5 TV/mL (25 replicates of each isolate per matrix). Sensitivity of the Aptima Trichomonas vaginalis 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.

Table 9: Microorganisms Tested in the Aptima Trichomonas vaginalis Assay

Microorganism	Concentration Tested		
	STM	PreservCyt	Urine
Acinetobacter lwoffi	$4.6x10^7$ CFU/mL	$4.6x10^7$ CFU/mL	$2.3x10^7$ CFU/mL
Actinomyces israelii	$2.1x10^8$ CFU/mL	$2.1x10^8$ CFU/mL	$1.1x10^8$ CFU/mL
Atopobium vaginae	$6.2x10^6$ CFU/mL	$6.2x10^6$ CFU/mL	$6.2x10^6$ CFU/mL
Bacteroides fragilis	$6.4x10^8$ CFU/mL	$6.4x10^8$ CFU/mL	$3.2x10^8$ CFU/mL
Bifidobacterium adolescentis	$7.2x10^7$ CFU/mL	$7.2x10^7$ CFU/mL	$3.6x10^7$ CFU/mL
Campylobacter jejuni	$7.2x10^7$ CFU/mL	$7.2x10^7$ CFU/mL	$3.6x10^7$ CFU/mL
Candida albicans	$1.2x10^8$ CFU/mL	$1.2x10^8$ CFU/mL	$5.9x10^7$ CFU/mL
Candida glabrata	$1.3x10^8$ CFU/mL	$1.4x10^8$ CFU/mL	$6.4x10^7$ CFU/mL
Candida parapsilosis	$9.2x10^7$ CFU/mL	$9.2x10^7$ CFU/mL	$4.6x10^7$ CFU/mL
Candida tropicalis	$1.8x10^7$ CFU/mL	$1.8x10^7$ CFU/mL	$9.1x10^6$ CFU/mL
Chlamydia trachomatis	$2.0x10^4$ TCID 50/mL	$2.0x10^4$ TCID 50/mL	$2.0x10^4$ TCID 50/mL
Clostridium difficile	$2.6x10^7$ CFU/mL	$2.6x10^7$ CFU/mL	$1.3x10^7$ CFU/mL
Clostridium perfringens	$1.9x10^8$ CFU/mL	$1.9x10^8$ CFU/mL	$9.4x10^7$ CFU/mL
Corynebacterium genitalium	$2.8x10^7$ CFU/mL	$2.8x10^7$ CFU/mL	$1.4x10^7$ CFU/mL
Cryptococcus neoformans	$5.8x10^7$ CFU/mL	$5.8x10^7$ CFU/mL	$2.9x10^7$ CFU/mL
Enterobacter aerogenes	$1.5x10^9$ CFU/mL	$1.5x10^9$ CFU/mL	$1.0x10^8$ CFU/mL
Enterococcus feacalis	$9.2x10^7$ CFU/mL	$9.2x10^7$ CFU/mL	$9.2x10^7$ CFU/mL
Escherichia coli	$2.2x10^8$ CFU/mL	$2.2x10^8$ CFU/mL	$2.2x10^8$ CFU/mL
Fusobacterium nucleatum	$1.3x10^8$ CFU/mL	$1.3x10^8$ CFU/mL	$6.4x10^7$ CFU/mL
Gardnerella vaginalis	$8.2x10^6$ CFU/mL	$8.2x10^6$ CFU/mL	$4.1x10^6$ CFU/mL
Haemophilus ducreyi	$2.1x10^9$ CFU/mL	$2.1x10^9$ CFU/mL	$3.1x10^9$ CFU/mL
Herpes simplex virus I	$2.0x10^5$ TCID 50/mL	$2.0x10^5$ TCID 50/mL	$2.0x10^5$ TCID 50/mL
Herpes simplex virus II	$2.0x10^5$ TCID 50/mL	$2.0x10^5$ TCID 50/mL	$2.0x10^5$ TCID 50/mL
HIV-1	$3.0x10^7$ copies/mL	$3.0x10^7$ copies/mL	$3.0x10^7$ copies/mL
HPV 16 (SiHa)	$1.0x10^5$ cell/mL	$1.0x10^5$ cells/mL	$1.0x10^5$ cells/mL
Klebsiella oxytoca	$9.6x10^8$CFU/mL	$9.6x10^8$ CFU/mL	$4.8x10^8$ CFU/mL
Lactobacillus acidophilus	$1.0x10^8$CFU/mL	$1.0x10^8$ CFU/mL	$5.2x10^7$ CFU/mL
Lactobacillus jensenii	$1.6x10^9$CFU/mL	$1.6x10^9$ CFU/mL	$8.2x10^8$ CFU/mL
Lactobacillus vaginalis	$4.6x10^8$CFU/mL	$4.6x10^8$ CFU/mL	$2.3x10^8$ CFU/mL
Listeria monocytogenes	$2.1x10^9$CFU/mL	$2.1x10^9$ CFU/mL	$1.0x10^9$ CFU/mL
Mobiluncus curtisii	$4.1x10^7$CFU/mL	$4.1x10^7$ CFU/mL	$4.1x10^7$ CFU/mL
Mycoplasma hominis	$1.0x10^8$CFU/mL	$1.0x10^8$ CFU/mL	$1.0x10^8$ CFU/mL
Neisseria gonorrhoeae	$2.7x10^8$ CFU/mL	$2.7x10^8$ CFU/mL	$1.4x10^8$ CFU/mL
Pentatrichomonas hominis	$2.2x10^6$ CFU/mL	$2.2x10^6$ CFU/mL	$1.3x10^6$ CFU/mL
Peptostreptococcus anaerobius	$2.2x10^8$ CFU/mL	$2.2x10^8$ CFU/mL	$1.1x10^8$ CFU/mL
Prevotella bivia	$5.2x10^8$ CFU/mL	$5.2x10^8$ CFU/mL	$2.6x10^8$ CFU/mL
Propionibacterium acnes	$1.6x10^8$ CFU/mL	$1.6x10^8$ CFU/mL	$1.6x10^8$ CFU/mL
Proteus mirabilis	$1.2x10^9$ CFU/mL	$1.2x10^9$ CFU/mL	$6.0x10^8$ CFU/mL
Pseudomonas aeruginosa	$1.5x10^8$ CFU/mL	1. $5x10^8$ CFU/mL	$1.5x10^8$ CFU/mL
Staphylococcus aureus	$2.8x10^8$ CFU/mL	$2.8x10^8$ CFU/mL	$2.8x10^8$ CFU/mL
Staphylococcus epidermidis	$3.0x10^8$ CFU/mL	$3.0x10^8$ CFU/mL	$1.5x10^8$ CFU/mL
Streptococcus agalactiae	$1.0x10^8$ CFU/mL	$1.0x10^8$ CFU/mL	$1.0x10^8$ CFU/mL
Streptococcus pyogenes	$1.0x10^8$ CFU/mL	$1.0x10^8$ CFU/mL	$8.9x10^7$ CFU/mL
Trichomonas tenax	$2.7x10^5$ CFU/mL	$2.7x10^5$ CFU/mL	$1.3x10^5$ CFU/mL
Ureaplasma urealyticum	$1.6x10^8$ CFU/mL	$1.4x10^8$ CFU/mL	$1.3x10^8$ CFU/mL

Interference

The following substances (at a concentration of 1% vol/vol or wt/vol) were individually spiked into STM, PreservCyt liquid Pap, and urine matrices and tested in the Aptima Trichomonas vaginalis Assay: over-the-counter personal lubricants, spermicides, deodorant sprays/powders, anti-fungal/ anti-itch medications, intravaginal hormones, porcine gastric mucus, glacial acetic acid, vinegar, and seminal fluid. Whole blood was tested at 10% vol/vol and KOVA-Trol I High Abnormal w/ Urobilinogen Urinalysis Control was substituted for urine to test for high levels of protein, glucose, ketones, bilirubin, nitrite, and urobilinogen. No interference was observed with any of the tested substances in the Aptima Trichomonas vaginalis 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).

Specimen Stability

Data to support the recommended shipping and storage conditions for the vaginal swab, PreservCyt liquid Pap, and urine specimens were generated with negative clinical specimens spiked with *T. vaginalis* to a final concentration of 250 TV/mL. Greater than 95% positivity was observed in all matrices (vaginal swab, PreservCyt liquid Pap, and urine) at all times and temperatures tested confirming the validity of the maximum storage times and temperatures described in *Specimen Collection and Storage*.

Panther System Assay Performance

Clinical Agreement Study

An agreement study between the Panther System and Tigris DTS System was conducted using residual specimens. Specimens were stored at -70°C for up to 18 months prior to being tested on the Panther System. A total of 2,082 specimens were tested at three sites using two lots of assay reagents and agreement with Tigris DTS System results was calculated. The 2,082 specimens consisted of 501 clinician collected vaginal swab specimens, 540 endocervical swab specimens, 495 female urine specimens, and 546 PreservCyt liquid Pap specimens. Of the 2,056 valid results the overall positive agreement between the Panther System and Tigris DTS System was 99.0%, the overall negative agreement was 99.2% and the overall combined agreement was 99.2%. The overall percent agreements by sample type along with the 95% confidence intervals are presented in Table 10. For all sample types except urine, the positive agreement between the two instrument platforms was 100%. With the urine specimen type the positive agreement between the Panther System and Tigris DTS System was 96.2%. The negative agreement between the instrument platforms was 99.1% for vaginal swabs, 98.1% for endocervical swabs, 100% for urine specimens, and 99.6% for PreservCyt specimens. The overall agreement between the Panther System and Tigris DTS System was 99.2% for vaginal swabs, 98.3% for endocervical swab, and 99.6% for urine and PreservCyt specimens.

Table 10: Clinical Specimen Agreement

	N	Tigris+ Panther+	Tigris+ Panther-	Tigris- Panther+	Tigris- Panther-	Positive Agreement (95% CI)	Negative Agreement (95% CI)	Overall Agreement (95% CI)
CVS	492	53	0	4	435	100% (93.2 - 100)	99.1% (97.7 - 99.6)	99.2% (97.9-99.7)
ES	525	48	0	9	468	100% (92.6 - 100)	98.1% (96.5 - 99.0)	98.3% (96.8-99.1)
Urine	495	50	2	0	443	96.2% (87.0 - 98.9)	100% (99.1 - 100)	99.6% (98.5-99.9)
PCyt	544	51	0	2	491	100% (93.0 - 100)	99.6% (98.5 - 99.9)	99.6% (98.7-99.9)
Overall	2056	202	2	15	1837	99.0% (96.5-99.7)	99.2% (98.7-99.5)	99.2% (98.7-99.5)

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

Assay Reproducibility

Aptima Trichomonas vaginalis Assay reproducibility was evaluated at three sites using the Panther System. Testing was performed over six days using two lots of assay reagents and six operators (two at each site). Reproducibility panels were created by spiking either urine matrix or PreservCyt Solution liquid Pap matrix with the appropriate amount of *T. vaginalis* lysate. Final *T. vaginalis* concentrations ranged from 0 to 1 TV/mL. Table 11 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 worklists, within worklists, and overall (total). Percent agreement with expected results is also shown. Samples with valid results were included in the analyses.

Table 11: Reproducibility Study: Reproducibility of the Aptima Trichomonas vaginalis Assay by Panel Member, Including Samples with Discordant Test Results

Conc Level	Target Conc[1]	N	Agreed	Agmt (%)	Mean RLU	Between Sites SD	Between Sites CV (%)	Between Operators SD	Between Operators CV (%)	Between Lots SD	Between Lots CV (%)	Between Runs SD	Between Runs CV (%)	Within Runs SD	Within Runs CV (%)	Total SD	Total CV (%)
PreservCyt Solution liquid Pap matrix																	
Neg	N/A	108	107	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	0.003	108	98	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	0.02	108	105	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	1	108	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																	
Neg	N/A	108	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	0.002	107	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	0.03	108	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	1	108	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.

[1]Concentration units = TV/mL.

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 was numerically negative. This occurred if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.

Analytical Sensitivity

Sensitivity panels containing 0.1 TV/mL in urine specimen matrix, PreservCyt liquid Pap specimen matrix, and vaginal swab matrix (120 replicates per matrix) were prepared with two strains of *T. vaginalis* (one Metronidazole-susceptible strain and one Metronidazole-resistant strain). Testing showed 100% positivity in all specimen matrices and in both strains of *T. vaginalis*.

Cross-Reactivity in the Presence of Microorganisms

Specificity

Specificity of the Aptima Trichomonas vaginalis Assay was evaluated by testing various microorganisms, including common flora of the genitourinary tract, opportunistic organisms, and closely related organisms. Testing was conducted in specimen transport medium (STM) with 25 replicates of each isolate. The list of organisms and the concentrations tested are provided in Table 12. No cross-reactivity or significant effect on Aptima Trichomonas vaginalis Assay specificity was observed with any of the organisms tested.

Sensitivity

Sensitivity of the Aptima Trichomonas vaginalis Assay was evaluated by testing the same organisms (Table 12) in STM spiked with *T. vaginalis* lysate to a final concentration of 2.5 TV/mL (25 replicates of each isolate). Sensitivity of the Aptima Trichomonas vaginalis 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.

Table 12: Microorganisms Tested in the Aptima Trichomonas vaginalis Assay on the Panther System

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	$1x10^6$ CFU/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 feacalis	$1x10^6$ 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 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-STM (10% final concentration).

No interference was observed with any of the tested substances in the Aptima Trichomonas vaginalis 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).

Carryover 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 batch of Aptima Trichomonas vaginalis 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.

Bibliography

1. **Weinstock, H., S. Berman, and W. Cates Jr.** 2004. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect. Sex. Reprod. Health **36**(1):6-10.
2. **Soper, D.** 2004. Trichomoniasis: under control or undercontrolled? Am. J. Obstet. Gynecol. **190**(1):281-290.
3. **Cotch, M. F., J. G. Pastorek II, R. P. Nugent, S. L. Hillier, R. S. Gibbs, D. H. Martin, et al.** 1997. *Trichomonas vaginalis* associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. Sex. Transm. Dis. **24**:353-360.
4. **Sorvillo, F. J., A. Kovacs, P. Kerndt, A. Stek, L. Muderspach, and L. Sanchez-Keeland.** 1998. Risk factors for trichomoniasis among women with HIV infection at a public clinic in Los Angeles County; Implications for HIV prevention. Am. J. Trop. Med. Hyg. **58**:495-500.
5. **Niccolai, L. M., J. J. Kopicko, A. Kassie, H. Petros, R. A. Clark, and P. Kissinger.** 2000. Incidence and predictors of reinfection with *Trichomonas vaginalis* in HIV-infected women. Sex. Transm. Dis. **27**:284-288.
6. **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.
7. **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.

IVD

CE

EC REP

Emergo Europe
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Customer Support: +1 844 Hologic (+1 844 465 6442)
customersupport@hologic.com

Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information visit www.hologic.com.

502536EN Rev. 004

2017-05