

## Aptima® Trichomonas vaginalis Assay (Panther® System)

For *in vitro* diagnostic use.

For U.S. Export only.

|                                                                           |           |
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## **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 infection (STI) agent in the United States, with an estimated 6.9 million new cases occurring annually (1).

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 (2, 3).

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

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

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

### **Principles of the Procedure**

The Aptima TV Assay involves 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 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 HPA. A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer and are reported as Relative Light Units (RLU).

## Warnings and Precautions

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

## 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 spills of this fluid occur, 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 in 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 transported and stored in accordance with the package insert are valid for testing, even if the expiration date on the collection tube has passed.
- M. 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.
- N. 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.
- O. Avoid cross-contamination by discarding used materials without passing them over any other container.
- P. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.
- Q. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another during specimen handling in the laboratory. Change gloves if they come in contact with a specimen.
- R. 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. Prior to rejecting a swab transport tube with no swab, verify that it is not an Aptima® Specimen Transfer Tube as this specimen transport tube will not contain a swab.
- S. Upon piercing, liquid can discharge from Aptima transfer tube caps under certain conditions. Follow instructions in the *Panther System Test Procedure* to prevent this occurrence.

**Assay Related**

- 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. Aptima controls and assay fluids (Panther System) can be interchanged from different lot numbers.
- Y. Do not combine 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 hazard information.

**Note:** For information on any hazard and precautionary statements that may be associated with reagents, refer to the SDS on the Safety Data Sheet Library at [www.hologicds.com](http://www.hologicds.com). For more information on the symbols, refer to the symbol legend on [www.hologic.com/package-inserts](http://www.hologic.com/package-inserts).

| Canada Hazard Information                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <b>Selection Reagent</b><br><b>Boric Acid 1 - 5%</b><br><b>Triton X-100 1 - 5%</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|  | <b>DANGER</b><br>H315 - Causes skin irritation.<br>H360FD - May damage fertility. May damage the unborn child.<br>P264 - Wash face, hands and any exposed skin thoroughly after handling.<br>P302 + P352 - IF ON SKIN: Wash with plenty of water and soap.<br>P321 - Specific treatment (See supplemental first aid instructions on the SDS).<br>P332 + P313 - If skin irritation occurs: Get medical advice/attention.<br>P362 + P364 - Take off contaminated clothing and wash it before reuse.<br>P201 - Obtain special instructions before use.<br>P202 - Do not handle until all safety precautions have been read and understood.<br>P280 - Wear protective gloves/protective clothing/eye protection/face protection.<br>P308 + P313 - IF exposed or concerned: Get medical advice/attention.<br>P405 - Store locked up.<br>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                 | 2°C to 8°C       | N/A                      | N/A             |
| Enzyme Reagent                        | 2°C to 8°C       | N/A                      | N/A             |
| Probe Reagent                         | 2°C to 8°C       | N/A                      | N/A             |
| Target Capture Reagent B              | 2°C to 8°C       | N/A                      | N/A             |
| Amplification Reconstitution Solution | 2°C to 30°C      | 2°C to 8°C               | 60 days         |
| Enzyme Reconstitution Solution        | 2°C to 30°C      | 2°C to 8°C               | 60 days         |
| Probe Reconstitution Solution         | 2°C to 30°C      | 2°C to 8°C               | 60 days         |
| Selection Reagent                     | 2°C to 30°C      | 2°C to 30°C              | 60 days         |
| Target Capture Reagent                | 15°C to 30°C     | 15°C to 30°C             | 60 days         |
| Positive Control                      | 2°C to 8°C       | N/A                      | Single Use Vial |
| Negative Control                      | 2°C to 8°C       | N/A                      | Single Use Vial |

- B. If the Selection Reagent is stored refrigerated, let it come to room temperature before placing on the Panther System.
- C. Working Target Capture Reagent (wTCR) is stable for 60 days when stored at 15°C to 30°C. Do not refrigerate.
- D. After reconstitution, Amplification Reagent, Enzyme Reagent, and Probe Reagent are stable for 60 days when stored at 2°C to 8°C.
- 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 on-board the Panther System have 72 hours of on-board stability.
- H. Avoid cross-contamination during reagent handling and storage. Recap all reconstituted reagents with new reagent caps each time prior to storage.
- I. The Probe Reagent 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. 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. 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  $\leq -20^{\circ}\text{C}$  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  $\leq -20^{\circ}\text{C}$  for up to 24 months after transfer.

3. PreservCyt Solution Pap Specimens

- a. Transport and store the PreservCyt Solution specimen at 2°C to 30°C for up to 30 days.
- b. Specimens collected in PreservCyt Solution must be transferred into an Aptima specimen transfer tube according to the instructions in the Aptima Specimen Transfer Kit 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  $\leq -20^{\circ}\text{C}$  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, foil barrier, or cap.

**Note:** Any condition resulting in loss or evaporation of media during transport, handling, or storage may impact the ability to pipette multiple aliquots.

3. If assayed samples need to be frozen or shipped, remove penetrable cap and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained.

4. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 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. 303537)

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

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

| Symbol       | Component                                                                                                                                      | Quantity     |              |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------|--------------|--------------|
|              |                                                                                                                                                | 250-test kit | 100-test kit |
| <b>A</b>     | <b>Amplification Reagent</b><br><i>Primers and nucleotides dried in buffered solution containing &lt; 5% bulking agent.</i>                    | 1 vial       | 1 vial       |
| <b>E</b>     | <b>Enzyme Reagent</b><br><i>Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing &lt; 10% bulking reagent.</i> | 1 vial       | 1 vial       |
| <b>P</b>     | <b>Probe Reagent</b><br><i>Chemiluminescent DNA probes dried in succinate buffered solution containing &lt; 5% detergent.</i>                  | 1 vial       | 1 vial       |
| <b>TCR-B</b> | <b>Assay Target Capture Reagent B</b><br><i>Buffered solution containing &lt; 5% detergent.</i>                                                | 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 |
| <b>AR</b> | <b>Amplification Reconstitution Solution</b><br><i>Aqueous solution containing preservatives.</i>             | 1 x 27.7 mL  | 1 x 11.9 mL  |
| <b>ER</b> | <b>Enzyme Reconstitution Solution</b><br><i>HEPES buffered solution containing a surfactant and glycerol.</i> | 1 x 11.1 mL  | 1 x 6.3 mL   |
| <b>PR</b> | <b>Probe Reconstitution Solution</b><br><i>Succinate buffered solution containing &lt; 5% detergent.</i>      | 1 x 35.4 mL  | 1 x 15.2 mL  |
| <b>S</b>  | <b>Selection Reagent</b><br><i>600 mM borate buffered solution containing surfactant.</i>                     | 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)

|            |                                                                                                                |             |             |
|------------|----------------------------------------------------------------------------------------------------------------|-------------|-------------|
| <b>TCR</b> | <b>Target Capture Reagent</b><br><i>Buffered solution containing capture oligomers and magnetic particles.</i> | 1 x 54.0 mL | 1 x 26.0 mL |
|            | <b>Reconstitution Collars</b>                                                                                  | 3           | 3           |
|            | <b>Master Lot Barcode Sheet</b>                                                                                | 1 sheet     | 1 sheet     |

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

| Symbol    | Component                                                                                                                           | Quantity   |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>NC</b> | <b>Negative Control</b><br><i>Non-infectious non-target nucleic acid in a buffered solution containing &lt; 5% detergent.</i>       | 5 x 1.7 mL |
| <b>PC</b> | <b>Positive Control</b><br><i>Non-infectious Trichomonas vaginalis organisms in buffered solution containing &lt; 5% detergent.</i> | 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<br><i>(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)</i> | 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<br><i>contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects</i>               | 303096 (5000 tests)                                |
| Tips, 1000 µL filtered, conductive, liquid sensing, and disposable                                                      | 901121 (10612513 Tecan)<br>903031 (10612513 Tecan) |
| <i>Not all products are available in all regions. Contact your representative for region-specific information</i>       | MME-04134 (30180117 Tecan)<br>MME-04128            |

|                                                                                                  |                   |
|--------------------------------------------------------------------------------------------------|-------------------|
| Aptima Specimen Transfer Kit<br><i>for use with specimens in PreservCyt Solution</i>             | 301154C           |
| Aptima Specimen Transfer Kit — printable<br><i>for use with specimens in PreservCyt Solution</i> | PRD-05110         |
| Aptima Multitest Swab Specimen Collection Kit                                                    | PRD-03546         |
| Aptima Unisex Swab Specimen Collection Kit for Endocervical<br>and Male Urethral Swab Specimens  | 301041            |
| Aptima Urine Specimen Collection Kit for Male and Female<br>Urine Specimens                      | 301040            |
| Aptima® Urine Specimen Transport Tubes for Male and Female<br>Urine Specimens                    | 105575            |
| Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite<br>solution                            | —                 |
| Disposable gloves                                                                                | —                 |
| Aptima penetrable caps                                                                           | 105668            |
| Replacement non-penetrable caps                                                                  | 103036A           |
| Replacement Caps for the 250-test kits                                                           | —                 |
| <i>Amplification and Probe reagent reconstitution<br/>    solutions</i>                          | CL0041 (100 caps) |
| <i>Enzyme Reagent reconstitution solution</i>                                                    | 501616 (100 caps) |
| <i>TCR and Selection reagent</i>                                                                 | CL0040 (100 caps) |
| Replacement Caps for 100-test kits                                                               | —                 |
| <i>Amplification, Enzyme, and Probe reagent<br/>    reconstitution solutions</i>                 | CL0041 (100 caps) |
| <i>TCR and Selection reagent</i>                                                                 | 501604 (100 caps) |

## Optional Materials

|                                                                                                |          |
|------------------------------------------------------------------------------------------------|----------|
|                                                                                                | Cat. No. |
| Aptima Trichomonas vaginalis Controls Kit                                                      | 302807   |
| Hologic® Bleach Enhancer for Cleaning<br><i>for routine cleaning of surfaces and equipment</i> | 302101   |
| Tube Rocker                                                                                    | —        |

## Panther System Test Procedure

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

### A. Work Area Preparation

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

### B. Reagent Reconstitution/Preparation of a New Kit

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

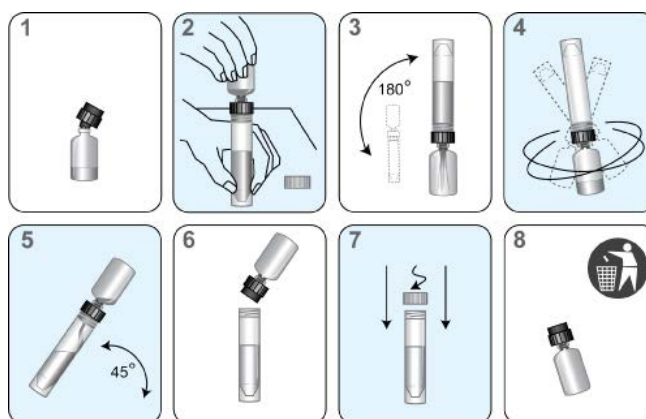
1. Prior to testing, Amplification, Enzyme, and Probe Reagents must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
  - a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.
  - b. Pair each reconstitution solution with its lyophilized reagent. Before attaching the reconstitution collar, ensure that the reconstitution solution and reagent have matching label symbols.
  - c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired. Label caps of reconstitution solution bottles.
  - d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 1, Step 1).
  - e. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
  - f. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 1, Step 2).
  - g. Slowly invert the assembled bottles. Allow the solution to drain from the reconstitution solution bottle into the glass vial (Figure 1, Step 3).
  - h. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (Figure 1, Step 4).
  - i. Wait 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.
  - j. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

- k. Recap the plastic bottle with either the saved labeled cap that corresponds to the reagent or new cap. Do not mismatch caps. Record the operator initials and reconstitution date on the label (Figure 1, Step 7).
- l. Discard the reconstitution collar and glass vial (Figure 1, Step 8).
- m. Thoroughly mix each reagent by gently inverting prior to loading onto the Panther System.

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

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

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



**Figure 1. Panther System Reconstitution Process**

2. Prepare Working Target Capture Reagent (wTCR)
  - 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 for a minimum of 25 minutes to ensure 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. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.

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

D. Control Preparation

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

E. Specimen Handling

1. Visually confirm that each specimen tube meets one of the following criteria.
  - a. The absence of a swab in the Aptima specimen transport tube for PreservCyt Solution Pap specimens.
  - 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 presence of a single blue Aptima collection swab in a unisex swab specimen transport tube.
2. Allow the specimens to reach room temperature (15°C to 30°C) prior to processing.

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

3. Inspect specimen tubes before loading into rack.
  - a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
  - b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.

- 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 3a–c may result in liquid discharge from the specimen tube cap.

**Note:** Up to 5 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 5 aliquots from the specimen tube can lead to processing errors.

#### F. System Preparation

1. Set up the system according to the instructions in the *Panther 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 Panther System software using the ATV Assay protocol, one pair of controls is required. The Positive Control and Negative Control can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Patient specimen pipetting will begin when one of the following two conditions has been met:
  - a. A pair of controls is currently being processed by the system.
  - b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours **unless**:
  - a. Controls results are invalid.
  - b. The associated assay reagent kit is removed from the system.
  - c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

#### B. Temperature

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

#### C. Glove Powder

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

#### D. Lab Contamination Monitoring Protocol for the Panther System

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

To monitor for laboratory contamination, the following procedure may be performed using the Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens or the Aptima Multitest Swab Specimen Collection Kit:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing if using the Aptima Unisex Swab Specimen Collection Kit) 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 Panther System software using the Aptima TV Assay protocol. 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. If the result is invalid upon retest, a new specimen should be collected. Report the first valid result. If the result is invalid upon retest, a new specimen should be collected.

| <b>Test Interpretation</b> | <b>Total RLU (x1,000)</b> |
|----------------------------|---------------------------|
| Negative                   | 0* to < 100               |
| Positive                   | 100 to < 2,400            |
| Invalid                    | 0* or ≥ 2,400             |

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

### **B. Quality Control Results and Acceptability**

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

The Controls must produce the following test results:

| <b>Control</b>     | <b>Total RLU (x1,000)</b> | <b><i>T. vaginalis</i> Result</b> |
|--------------------|---------------------------|-----------------------------------|
| NC Control – TRICH | 0* and < 20               | Negative                          |
| PC Control + TRICH | ≥ 500 and < 2,400         | 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.

## **Limitations**

- A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this insert may result in erroneous results.
- B. The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of *T. vaginalis*.
- C. TV-positive mucoid samples may exhibit decreased RLU values. To ensure proper endocervical sampling, excess mucus should be removed.
- D. Urine, vaginal swab and PreservCyt Solution 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 the ThinPrep® 2000 System or the ThinPrep® 3000 System has not been evaluated with the Aptima TV Assay.
- N. The performance of the Panther System has not been determined at altitudes above 2,000 m (6,561 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.

## Panther System Expected Values

### Prevalence

Estimates of the prevalence of *T. vaginalis* in different populations depend on the sensitivity of the test in detecting the infection and on patient risk factors such as age, lifestyle, and the presence or absence of symptoms. A summary of the positivity of *T. vaginalis* as determined by the Aptima TV Assay during the Panther System clinical studies is shown in Table 1 and Table 2 for the two multicenter clinical studies by clinical site and overall.

Table 1: Prevalence of *T. vaginalis* as Determined by the Aptima TV 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 TV Assay in Patient-collected Vaginal Swab, Female Urine, and Male Urine Samples by Clinical Site (Clinical Study 2)

| Site | Positivity % (# positive / # tested with valid results) |                 |                |
|------|---------------------------------------------------------|-----------------|----------------|
|      | PVS                                                     | FU              | MU             |
| 1    | 0 (0/16)                                                | 0 (0/16)        | 0 (0/180)      |
| 2    | 11.1 (36/325)                                           | 10.4 (38/364)   | 4.4 (16/364)   |
| 3    | 8.5 (6/71)                                              | 9.5 (7/74)      | 1.7 (1/60)     |
| 4    | NC (0/0)                                                | NC (0/0)        | 0 (0/13)       |
| 5    | 8.8 (15/170)                                            | 8.8 (15/171)    | 2.9 (12/407)   |
| 6    | 5.8 (24/416)                                            | 5.8 (24/413)    | 0.7 (2/304)    |
| 7    | 6.1 (11/179)                                            | 5.3 (10/187)    | 1.3 (3/225)    |
| 8    | 0 (0/38)                                                | 0 (0/39)        | 0 (0/32)       |
| 9    | 10.8 (32/297)                                           | 9.8 (25/255)    | 2.4 (5/210)    |
| 10   | 20.2 (37/183)                                           | 19.8 (36/182)   | 6.7 (6/89)     |
| 11   | 6.7 (6/90)                                              | 3.7 (3/81)      | 0 (0/51)       |
| All  | 9.4 (167/1,785)                                         | 8.9 (158/1,782) | 2.3 (45/1,935) |

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 TV 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 TV Assay by Specimen Type (Clinical Study 2)

| Specimen Type | Prevalence (%) | PPV (%) | NPV (%) |
|---------------|----------------|---------|---------|
| <b>PVS</b>    | 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    |
| <b>FU</b>     | 1              | 100     | 100     |
|               | 2              | 100     | 100     |
|               | 5              | 100     | 100     |
|               | 10             | 100     | 100     |
|               | 15             | 100     | 100     |
|               | 20             | 100     | 100     |
|               | 25             | 100     | 100     |
| <b>MU</b>     | 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**

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, Female Urine Samples, 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 STI 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.

### **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, multi-center 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, 5,922 were processed in valid Aptima TV Assay runs. Of these, 5,833 (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 2 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 5,502 specimens from 3,820 evaluable subjects were included in the analyses comparing Aptima TV Assay results to the specimen-specific CCA interpretation: 1,785 patient-collected vaginal swab, 1,782 female urine, and 1,935 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 for Clinical Study 1, and Table 8 and Table 9 for Clinical Study 2. The infected status algorithm differed among the two studies.

Table 8 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 each specimen type by symptom status and overall. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. Prevalence was higher in all women subjects.

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

| Specimen Type | Symptom Status | n   | TP | FP <sup>1</sup> | TN  | FN <sup>2</sup> | Prev % | Sensitivity % (95% CI) <sup>3</sup> | Specificity % (95% CI) <sup>3</sup> | PPV % (95% CI) <sup>4</sup> | NPV % (95% CI) <sup>4</sup> |
|---------------|----------------|-----|----|-----------------|-----|-----------------|--------|-------------------------------------|-------------------------------------|-----------------------------|-----------------------------|
| FU            | Asymptomatic   | 279 | 13 | 1 <sup>a</sup>  | 263 | 2 <sup>m</sup>  | 5.4    | 86.7<br>(62.1–96.3)                 | 99.6<br>(97.9–99.9)                 | 92.9<br>(71.6–99.8)         | 99.2<br>(97.8–99.9)         |
|               | Symptomatic    | 361 | 46 | 4 <sup>b</sup>  | 309 | 2 <sup>n</sup>  | 13.3   | 95.8<br>(86.0–98.8)                 | 98.7<br>(96.8–99.5)                 | 92.0<br>(82.4–97.5)         | 99.4<br>(97.9–99.9)         |
|               | All            | 640 | 59 | 5 <sup>c</sup>  | 572 | 4 <sup>o</sup>  | 9.8    | 93.7<br>(84.8–97.5)                 | 99.1<br>(98.0–99.6)                 | 92.2<br>(84.0–97.1)         | 99.3<br>(98.3–99.8)         |
| CVS           | Asymptomatic   | 274 | 12 | 7 <sup>d</sup>  | 255 | 0               | 4.4    | 100<br>(75.8–100)                   | 97.3<br>(94.6–98.7)                 | 63.2<br>(45.8–80.9)         | 100<br>(98.8–100)           |
|               | Symptomatic    | 393 | 57 | 4 <sup>e</sup>  | 332 | 0               | 14.5   | 100<br>(93.7–100)                   | 98.8<br>(97.0–99.5)                 | 93.4<br>(84.9–98.1)         | 100<br>(98.9–100)           |
|               | All            | 667 | 69 | 11 <sup>f</sup> | 587 | 0               | 10.3   | 100<br>(94.7–100)                   | 98.2<br>(96.7–99.0)                 | 86.3<br>(77.9–92.6)         | 100<br>(99.4–100)           |
| ES            | Asymptomatic   | 309 | 16 | 5 <sup>g</sup>  | 288 | 0               | 5.2    | 100<br>(80.6–100)                   | 98.3<br>(96.1–99.3)                 | 76.2<br>(58.1–90.8)         | 100<br>(98.9–100)           |
|               | Symptomatic    | 391 | 51 | 7 <sup>h</sup>  | 333 | 0               | 13.0   | 100<br>(93.0–100)                   | 97.9<br>(95.8–99.0)                 | 87.9<br>(78.1–94.7)         | 100<br>(99.0–100)           |
|               | All            | 700 | 67 | 12 <sup>i</sup> | 621 | 0               | 9.6    | 100<br>(94.6–100)                   | 98.1<br>(96.7–98.9)                 | 84.8<br>(76.3–91.5)         | 100<br>(99.4–100)           |
| PCyt          | Asymptomatic   | 324 | 18 | 1 <sup>j</sup>  | 305 | 0               | 5.6    | 100<br>(82.4–100)                   | 99.7<br>(98.2–99.9)                 | 94.7<br>(76.5–99.9)         | 100<br>(98.9–100)           |
|               | Symptomatic    | 406 | 57 | 5 <sup>k</sup>  | 344 | 0               | 14.0   | 100<br>(93.7–100)                   | 98.6<br>(96.7–99.4)                 | 91.9<br>(83.1–97.2)         | 100<br>(99.0–100)           |
|               | All            | 730 | 75 | 6 <sup>l</sup>  | 649 | 0               | 10.3   | 100<br>(95.1–100)                   | 99.1<br>(98.0–99.6)                 | 92.6<br>(85.2–97.1)         | 100<br>(99.5–100)           |

CI = confidence interval; FU = female urine; 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.

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

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

<sup>3</sup>Score confidence interval.

<sup>4</sup>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 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 each specimen type by collection site. For each specimen type, performance was similar across collection sites. Prevalence varied across collection sites, as expected.

Table 6: Performance Characteristics of the Aptima TV Assay by Collection Site

| Site | Specimen Type | n   | TP | FP | TN  | FN | Prev % | Sensitivity (95% CI) <sup>1</sup> | Specificity (95% CI) <sup>1</sup> | PPV % (95% CI) <sup>2</sup> | NPV % (95% CI) <sup>2</sup> |
|------|---------------|-----|----|----|-----|----|--------|-----------------------------------|-----------------------------------|-----------------------------|-----------------------------|
| 1    | FU            | 52  | 8  | 0  | 42  | 2  | 19.2   | 80.0 (49.0–94.3)                  | 100 (91.6–100)                    | 100 (71.6–100)              | 95.5 (88.3–99.4)            |
|      | CVS           | 52  | 8  | 1  | 43  | 0  | 15.4   | 100 (67.6–100)                    | 97.7 (88.2–99.6)                  | 88.9 (60.2–99.7)            | 100 (93.7–100)              |
|      | ES            | 53  | 9  | 2  | 42  | 0  | 17.0   | 100 (70.1–100)                    | 95.5 (84.9–98.7)                  | 81.8 (56.9–97.4)            | 100 (93.5–100)              |
|      | PCyt          | 59  | 11 | 0  | 48  | 0  | 18.6   | 100 (74.1–100)                    | 100 (92.6–100)                    | 100 (75.6–100)              | 100 (93.9–100)              |
| 2    | FU            | 55  | 2  | 0  | 52  | 1  | 5.5    | 66.7 (20.8–93.9)                  | 100 (93.1–100)                    | 100 (29.4–100)              | 98.1 (95.0–100)             |
|      | CVS           | 52  | 3  | 1  | 48  | 0  | 5.8    | 100 (43.9–100)                    | 98.0 (89.3–99.6)                  | 75.0 (28.5–99.2)            | 100 (95.8–100)              |
|      | ES            | 56  | 4  | 1  | 51  | 0  | 7.1    | 100 (51.0–100)                    | 98.1 (89.9–99.7)                  | 80.0 (40.5–99.4)            | 100 (95.6–100)              |
|      | PCyt          | 63  | 5  | 0  | 58  | 0  | 7.9    | 100 (56.6–100)                    | 100 (93.8–100)                    | 100 (58.3–100)              | 100 (95.7–100)              |
| 3    | FU            | 13  | 1  | 1  | 11  | 0  | 7.7    | 100 (20.7–100)                    | 91.7 (64.6–98.5)                  | 50.0 (3.0–97.5)             | 100 (91.3–100)              |
|      | CVS           | 12  | 2  | 0  | 10  | 0  | 16.7   | 100 (34.2–100)                    | 100 (72.2–100)                    | 100 (32.1–100)              | 100 (85.6–100)              |
|      | ES            | 16  | 2  | 0  | 14  | 0  | 12.5   | 100 (34.2–100)                    | 100 (78.5–100)                    | 100 (31.5–100)              | 100 (89.3–100)              |
|      | PCyt          | 17  | 2  | 1  | 14  | 0  | 11.8   | 100 (34.2–100)                    | 93.3 (70.2–98.8)                  | 66.7 (19.9–98.8)            | 100 (89.5–100)              |
| 4    | FU            | 43  | 7  | 1  | 35  | 0  | 16.3   | 100 (64.6–100)                    | 97.2 (85.8–99.5)                  | 87.5 (57.2–99.6)            | 100 (92.6–100)              |
|      | CVS           | 41  | 7  | 1  | 33  | 0  | 17.1   | 100 (64.6–100)                    | 97.1 (85.1–99.5)                  | 87.5 (57.3–99.6)            | 100 (92.2–100)              |
|      | ES            | 41  | 7  | 0  | 34  | 0  | 17.1   | 100 (64.6–100)                    | 100 (89.8–100)                    | 100 (66.7–100)              | 100 (92.2–100)              |
|      | PCyt          | 43  | 7  | 1  | 35  | 0  | 16.3   | 100 (64.6–100)                    | 97.2 (85.8–99.5)                  | 87.5 (57.2–99.6)            | 100 (92.6–100)              |
| 5    | FU            | 136 | 1  | 0  | 135 | 0  | 0.7    | 100 (20.7–100)                    | 100 (97.2–100)                    | 100 (6.4–100)               | 100 (99.3–100)              |
|      | CVS           | 145 | 1  | 0  | 144 | 0  | 0.7    | 100 (20.7–100)                    | 100 (97.4–100)                    | 100 (6.4–100)               | 100 (99.3–100)              |
|      | ES            | 162 | 1  | 0  | 161 | 0  | 0.6    | 100 (20.7–100)                    | 100 (97.7–100)                    | 100 (6.4–100)               | 100 (99.4–100)              |
|      | PCyt          | 167 | 1  | 0  | 166 | 0  | 0.6    | 100 (20.7–100)                    | 100 (97.7–100)                    | 100 (6.4–100)               | 100 (99.4–100)              |
| 6    | FU            | 70  | 9  | 1  | 59  | 1  | 14.3   | 90.0 (59.6–98.2)                  | 98.3 (91.1–99.7)                  | 90.0 (63.8–99.6)            | 98.3 (93.1–100)             |
|      | CVS           | 67  | 10 | 2  | 55  | 0  | 14.9   | 100 (72.2–100)                    | 96.5 (88.1–99.0)                  | 83.3 (59.2–98.2)            | 100 (94.8–100)              |
|      | ES            | 80  | 13 | 4  | 63  | 0  | 16.3   | 100 (77.2–100)                    | 94.0 (85.6–97.7)                  | 76.5 (57.1–92.2)            | 100 (95.3–100)              |
|      | PCyt          | 79  | 15 | 2  | 62  | 0  | 19.0   | 100 (79.6–100)                    | 96.9 (89.3–99.1)                  | 88.2 (68.4–98.4)            | 100 (95.1–100)              |
| 7    | FU            | 142 | 11 | 0  | 131 | 0  | 7.7    | 100 (74.1–100)                    | 100 (97.2–100)                    | 100 (75.1–100)              | 100 (97.7–100)              |
|      | CVS           | 173 | 18 | 3  | 152 | 0  | 10.4   | 100 (82.4–100)                    | 98.1 (94.5–99.3)                  | 85.7 (67.7–96.7)            | 100 (97.9–100)              |
|      | ES            | 161 | 12 | 3  | 146 | 0  | 7.5    | 100 (75.8–100)                    | 98.0 (94.2–99.3)                  | 80.0 (58.3–95.4)            | 100 (97.9–100)              |
|      | PCyt          | 168 | 15 | 1  | 152 | 0  | 8.9    | 100 (79.6–100)                    | 99.3 (96.4–99.9)                  | 93.8 (73.2–99.8)            | 100 (97.9–100)              |
| 8    | FU            | 82  | 10 | 1  | 71  | 0  | 12.2   | 100 (72.2–100)                    | 98.6 (92.5–99.8)                  | 90.9 (64.9–99.7)            | 100 (95.9–100)              |
|      | CVS           | 80  | 10 | 2  | 68  | 0  | 12.5   | 100 (72.2–100)                    | 97.1 (90.2–99.2)                  | 83.3 (59.0–98.2)            | 100 (95.8–100)              |
|      | ES            | 83  | 9  | 2  | 72  | 0  | 10.8   | 100 (70.1–100)                    | 97.3 (90.7–99.3)                  | 81.8 (56.3–97.4)            | 100 (96.1–100)              |
|      | PCyt          | 86  | 9  | 0  | 77  | 0  | 10.5   | 100 (70.1–100)                    | 100 (95.2–100)                    | 100 (71.4–100)              | 100 (96.2–100)              |
| 9    | FU            | 47  | 10 | 1  | 36  | 0  | 21.3   | 100 (72.2–100)                    | 97.3 (86.2–99.5)                  | 90.9 (65.6–99.7)            | 100 (92.3–100)              |
|      | CVS           | 45  | 10 | 1  | 34  | 0  | 22.2   | 100 (72.2–100)                    | 97.1 (85.5–99.5)                  | 90.9 (65.7–99.7)            | 100 (91.9–100)              |
|      | ES            | 48  | 10 | 0  | 38  | 0  | 20.8   | 100 (72.2–100)                    | 100 (90.8–100)                    | 100 (74.0–100)              | 100 (92.5–100)              |
|      | PCyt          | 48  | 10 | 1  | 37  | 0  | 20.8   | 100 (72.2–100)                    | 97.4 (86.5–99.5)                  | 90.9 (65.6–99.7)            | 100 (92.5–100)              |

CI = confidence interval; FU = female urine; 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.

<sup>1</sup>Score confidence interval.

<sup>2</sup>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 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: Performance Characteristics of the Aptima TV Assay in PreservCyt Solution Pap Specimens by Collection Device Type**

| Collection Device <sup>1</sup> | n   | TP | FP | TN  | FN | Prev % | Sensitivity (95% CI) <sup>2</sup> | Specificity (95% CI) <sup>2</sup> | PPV % (95% CI) <sup>3</sup> | NPV % (95% CI) <sup>3</sup> |
|--------------------------------|-----|----|----|-----|----|--------|-----------------------------------|-----------------------------------|-----------------------------|-----------------------------|
| 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; PPV = positive predictive value; NPV = negative predictive value.

<sup>1</sup>All results are from Clinical Study 1.

<sup>2</sup>Score confidence interval.

<sup>3</sup>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 8 shows the positive (PPA) and negative (NPA) percent agreement of the assay in female patient-collected vaginal swab, and female and male urine specimens based on specimen-specific comparison. Prevalence was higher in symptomatic subjects.

**Table 8: 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 <sup>1</sup> | n     | TP  | FP             | TN    | FN <sup>3</sup> | Prev % | PPA % (95% CI) <sup>4</sup> | NPA % (95% CI) <sup>4</sup> |
|---------------|-----------------------------|-------|-----|----------------|-------|-----------------|--------|-----------------------------|-----------------------------|
| PVS           | Asymptomatic                | 932   | 59  | 3 <sup>a</sup> | 868   | 2 <sup>a</sup>  | 6.5    | 96.7 (88.8–99.1)            | 99.7 (99.0–99.9)            |
|               | Symptomatic                 | 853   | 99  | 6 <sup>a</sup> | 748   | 0               | 11.6   | 100 (96.3–100)              | 99.2 (98.3–99.6)            |
|               | All                         | 1,785 | 158 | 9              | 1,616 | 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                         | 1,782 | 158 | 0              | 1,624 | 0               | 8.9    | 100 (97.6–100)              | 100 (99.8–100)              |
| MU            | Asymptomatic                | 1125  | 21  | 1 <sup>b</sup> | 1,103 | 0               | 1.9    | 100 (84.5–100)              | 99.9 (99.5–100)             |
|               | Symptomatic                 | 810   | 21  | 2 <sup>c</sup> | 787   | 0               | 2.6    | 100 (84.5–100)              | 99.7 (99.1–99.9)            |
|               | All                         | 1,935 | 42  | 3              | 1,890 | 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.

<sup>1</sup>Patient-collected vaginal swab, female urine and male urine sample results are from Clinical Study 2.

<sup>2</sup>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): <sup>a</sup>No discordant resolution testing result was available for PVS samples; <sup>b</sup>0/1; <sup>c</sup>0/1 (no discordant resolution testing result was available for 1 sample).

<sup>3</sup>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): <sup>a</sup>No discordant resolution testing result was available for PVS samples.

<sup>4</sup>Score CI.

Table 9 shows the PPA and NPA of the assay in female patient-collected vaginal swab, and female and male urine specimens by collection site.

**Table 9: Performance Characteristics of the Aptima TV Assay in Female Patient-collected Vaginal Swab, and Male and Female Urine Specimens by Collection Site, Compared to CCA**

| Site | Specimen Type <sup>1</sup> | n   | TP  | FP  | TN  | FN  | Prev % | PPA % (95% CI)   | NPA % (95% CI) <sup>2</sup> |
|------|----------------------------|-----|-----|-----|-----|-----|--------|------------------|-----------------------------|
| 1    | PVS                        | 16  | 0   | 0   | 16  | 0   | 0.0    | NC               | 100 (80.6–100)              |
|      | FU                         | 16  | 0   | 0   | 16  | 0   | 0.0    | NC               | 100 (80.6–100)              |
|      | MU                         | 180 | 0   | 0   | 180 | 0   | 0.0    | NC               | 100 (97.9–100)              |
| 2    | PVS                        | 325 | 31  | 5   | 288 | 1   | 9.8    | 96.9 (84.3–99.4) | 98.3 (96.1–99.3)            |
|      | FU                         | 364 | 38  | 0   | 326 | 0   | 10.4   | 100 (90.8–100)   | 100 (98.8–100)              |
|      | MU                         | 364 | 16  | 0   | 348 | 0   | 4.4    | 100 (80.6–100)   | 100 (98.9–100)              |
| 3    | PVS                        | 71  | 6   | 0   | 65  | 0   | 8.5    | 100 (61.0–100)   | 100 (94.4–100)              |
|      | FU                         | 74  | 7   | 0   | 67  | 0   | 9.5    | 100 (64.6–100)   | 100 (94.6–100)              |
|      | MU                         | 60  | 1   | 0   | 59  | 0   | 1.7    | 100 (20.7–100)   | 100 (93.9–100)              |
| 4    | PVS <sup>3</sup>           | N/A | N/A | N/A | N/A | N/A | N/A    | N/A              | N/A                         |
|      | FU <sup>3</sup>            | N/A | N/A | N/A | N/A | N/A | N/A    | N/A              | N/A                         |
|      | MU                         | 13  | 0   | 0   | 13  | 0   | 0.0    | NC               | 100 (77.2–100)              |
| 5    | PVS                        | 170 | 15  | 0   | 155 | 0   | 8.8    | 100 (79.6–100)   | 100 (97.6–100)              |
|      | FU                         | 171 | 15  | 0   | 156 | 0   | 8.8    | 100 (79.6–100)   | 100 (97.6–100)              |
|      | MU                         | 407 | 11  | 1   | 395 | 0   | 2.7    | 100 (74.1–100)   | 99.7 (98.6–100)             |
| 6    | PVS                        | 416 | 24  | 0   | 392 | 0   | 5.8    | 100 (86.2–100)   | 100 (99.0–100)              |
|      | FU                         | 413 | 24  | 0   | 389 | 0   | 5.8    | 100 (86.2–100)   | 100 (99.0–100)              |
|      | MU                         | 304 | 2   | 0   | 302 | 0   | 0.7    | 100 (34.2–100)   | 100 (98.7–100)              |
| 7    | PVS                        | 179 | 11  | 0   | 168 | 0   | 6.1    | 100 (74.1–100)   | 100 (97.8–100)              |
|      | FU                         | 187 | 10  | 0   | 177 | 0   | 5.3    | 100 (72.2–100)   | 100 (97.9–100)              |
|      | MU                         | 225 | 2   | 1   | 222 | 0   | 0.9    | 100 (34.2–100)   | 99.6 (97.5–99.9)            |
| 8    | PVS                        | 38  | 0   | 0   | 38  | 0   | 0.0    | NC               | 100 (90.8–100)              |
|      | FU                         | 39  | 0   | 0   | 39  | 0   | 0.0    | NC               | 100 (91.0–100)              |
|      | MU                         | 32  | 0   | 0   | 32  | 0   | 0.0    | NC               | 100 (89.3–100)              |
| 9    | PVS                        | 297 | 28  | 4   | 265 | 0   | 9.4    | 100 (87.9–100)   | 98.5 (96.2–99.4)            |
|      | FU                         | 255 | 25  | 0   | 230 | 0   | 9.8    | 100 (86.7–100)   | 100 (98.4–100)              |
|      | MU                         | 210 | 5   | 0   | 205 | 0   | 2.4    | 100 (56.6–100)   | 100 (98.2–100)              |
| 10   | PVS                        | 183 | 37  | 0   | 145 | 1   | 20.8   | 97.4 (86.5–99.5) | 100 (97.4–100)              |
|      | FU                         | 182 | 36  | 0   | 146 | 0   | 19.8   | 100 (90.4–100)   | 100 (97.4–100)              |
|      | MU                         | 89  | 5   | 1   | 83  | 0   | 5.6    | 100 (56.6–100)   | 98.8 (93.6–99.8)            |
| 11   | PVS                        | 90  | 6   | 0   | 84  | 0   | 6.7    | 100 (61.0–100)   | 100 (95.6–100)              |
|      | FU                         | 81  | 3   | 0   | 78  | 0   | 3.7    | 100 (43.9–100)   | 100 (95.3–100)              |
|      | MU                         | 51  | 0   | 0   | 51  | 0   | 0.0    | NC               | 100 (93.0–100)              |

CI = confidence interval; FN = false negative; FP = false positive; FM = female urine; MU = male urine;

N/A = not applicable; NC = not calculable; NPA = negative percent agreement; PPA = positive percent agreement;

Prev = prevalence; PVS = patient-collected vaginal swab; TN = true negative; TP = true positive.

<sup>1</sup>All results are from Clinical Study 2.

<sup>2</sup>Score CI.

<sup>3</sup>There were no female subjects evaluable for either patient-collected vaginal swab or female urine specimens at this site.

## **RLU Distribution of Aptima Trichomonas vaginalis Controls**

The distribution of the RLU values for the Aptima TV Assay controls is presented in Table 10 for all valid Aptima TV Assay runs performed during Clinical Study 1 and Clinical Study 2.

Table 10: RLU Distribution of Aptima TV Negative and Positive Controls

| Control  | Statistic | Total RLU (x1,000) |                  |
|----------|-----------|--------------------|------------------|
|          |           | 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      | 1,262.3            | NC               |
|          | SD        | 45.89              | NC               |
|          | Median    | 1,276.0            | 1,400.0          |
|          | Minimum   | 1,168              | 1157             |
|          | Maximum   | 1,322              | 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 1,000 with the digits after the decimal point truncated.

## **Reproducibility Study**

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

Reproducibility panel members were created by using negative urine specimens in Aptima Urine Transport Medium (UTM) or negative PreservCyt Solution Pap specimens with STM. 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 TV/mL 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 runs,

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

Table 11: Aptima TV 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      | 1,185.5  | 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      | 1,208.3  | 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 1,000 with the digits after the decimal point truncated.

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

## **Panther System Analytical Performance**

### **Analytical Sensitivity**

Sensitivity panels containing 0.1 TV/mL in urine specimen matrix, PreservCyt Solution 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 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 and PreservCyt Solution in 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 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 12) in STM and PreservCyt Solution in STM spiked with *T. vaginalis* lysate 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.

Table 12: Microorganisms Tested in the Aptima TV Assay

| Microorganism                       | Concentration                            | Microorganism                    | Concentration                  |
|-------------------------------------|------------------------------------------|----------------------------------|--------------------------------|
| <i>Acinetobacter lwoffii</i>        | 1x10 <sup>6</sup> CFU/mL                 | HPV 16                           | 2.5x10 <sup>6</sup> copies/mL  |
| <i>Actinomyces israelii</i>         | 1x10 <sup>6</sup> CFU/mL                 | HPV 6                            | 2.5x10 <sup>6</sup> copies/mL  |
| <i>Atopobium vaginae</i>            | 1x10 <sup>6</sup> CFU/mL                 | <i>Klebsiella pneumoniae</i>     | 1x10 <sup>6</sup> CFU/mL       |
| <i>Bacteroides fragilis</i>         | 1x10 <sup>6</sup> CFU/mL                 | <i>Lactobacillus acidophilus</i> | 1x10 <sup>6</sup> CFU/mL       |
| <i>Bifidobacterium adolescentis</i> | 1x10 <sup>6</sup> CFU/mL                 | <i>Lactobacillus crispatus</i>   | 1x10 <sup>6</sup> CFU/mL       |
| <i>Campylobacter jejuni</i>         | 1x10 <sup>6</sup> CFU/mL                 | <i>Listeria monocytogenes</i>    | 1x10 <sup>6</sup> CFU/mL       |
| <i>Candida albicans</i>             | 1x10 <sup>6</sup> CFU/mL                 | <i>Mobiluncus curtisii</i>       | 1x10 <sup>6</sup> CFU/mL       |
| <i>Chlamydia trachomatis</i>        | 1x10 <sup>6</sup> IFU/mL                 | <i>Mycoplasma genitalium</i>     | 2.5 x10 <sup>6</sup> copies/mL |
| <i>Clostridium difficile</i>        | 1x10 <sup>6</sup> CFU/mL                 | <i>Mycoplasma hominis</i>        | 1x10 <sup>6</sup> CFU/mL       |
| <i>Corynebacterium genitalium</i>   | 1x10 <sup>6</sup> CFU/mL                 | <i>Neisseria gonorrhoeae</i>     | 1x10 <sup>6</sup> CFU/mL       |
| <i>Cryptococcus neoformans</i>      | 1x10 <sup>6</sup> CFU/mL                 | <i>Pentatrichomonas hominis</i>  | 1x10 <sup>6</sup> cells/mL     |
| Cytomegalovirus                     | 2x10 <sup>5</sup> TCID <sub>50</sub> /mL | <i>Peptostreptococcus magnus</i> | 1x10 <sup>6</sup> CFU/mL       |
| <i>Dientamoeba fragilis</i>         | 1x10 <sup>6</sup> CFU/mL                 | <i>Prevotella bivia</i>          | 1x10 <sup>6</sup> CFU/mL       |
| <i>Enterobacter cloacae</i>         | 1x10 <sup>6</sup> CFU/mL                 | <i>Propionibacterium acnes</i>   | 1x10 <sup>6</sup> CFU/mL       |
| <i>Enterococcus faecalis</i>        | 1x10 <sup>6</sup> CFU/mL                 | <i>Proteus vulgaris</i>          | 1x10 <sup>6</sup> CFU/mL       |
| <i>Escherichia coli</i>             | 1x10 <sup>6</sup> CFU/mL                 | <i>Pseudomonas aeruginosa</i>    | 1x10 <sup>6</sup> CFU/mL       |

Table 12: Microorganisms Tested in the Aptima TV Assay (continued)

| Microorganism                | Concentration                            | Microorganism                     | Concentration              |
|------------------------------|------------------------------------------|-----------------------------------|----------------------------|
| <i>Gardnerella vaginalis</i> | 1x10 <sup>6</sup> CFU/mL                 | <i>Staphylococcus aureus</i>      | 1x10 <sup>6</sup> CFU/mL   |
| <i>Haemophilus ducreyi</i>   | 1x10 <sup>6</sup> CFU/mL                 | <i>Staphylococcus epidermidis</i> | 1x10 <sup>6</sup> CFU/mL   |
| Herpes simplex virus I       | 2x10 <sup>5</sup> TCID <sub>50</sub> /mL | <i>Streptococcus agalactiae</i>   | 1x10 <sup>6</sup> CFU/mL   |
| Herpes simplex virus II      | 2x10 <sup>5</sup> TCID <sub>50</sub> /mL | <i>Trichomonas tenax</i>          | 1x10 <sup>6</sup> cells/mL |
| HIV-1                        | 2.5x10 <sup>6</sup> copies/mL            | <i>Ureaplasma urealyticum</i>     | 1x10 <sup>6</sup> CFU/mL   |

TCID<sub>50</sub> = Tissue culture infection dose<sub>50</sub>; CFU = Colony forming units.

## Interference

The following substances were individually spiked into STM, urine in UTM, and PreservCyt Solution in STM for a final concentration of 1% (vol/vol or wt/vol): personal lubricants, personal deodorants, spermicides, anti-fungals, intravaginal hormones, porcine gastric mucus, seminal fluid from 25 donors, and whole blood (10% final concentration).

The effects of urine metabolites were tested by the addition of KOVA-Trol® I High Abnormal w/ Urobilinogen Urinalysis Control diluted into 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).

## 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.

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