Aptima Mycoplasma genitalium Assay

For *in vitro* diagnostic use.

General Information

Intended Use

The Aptima Mycoplasma genitalium assay is an *in vitro* nucleic acid amplification test (NAAT) for the qualitative detection of ribosomal RNA (rRNA) from *Mycoplasma genitalium* on the fully automated Panther system. It is intended for use as an aid in the diagnosis of *M. genitalium* urogenital infections in male and female patients.

The assay may be used to test: clinician-collected and self-collected vaginal swab specimens, clinician-collected endocervical swab specimens, clinician-collected cervical specimens collected in PreservCyt™ solution, self-collected first-catch male and female urine specimens, clinician-collected male urethral swab specimens, and self-collected penile meatal swab specimens.

Summary and Explanation of the Test

M. genitalium is a sexually-transmitted gram-negative bacterium belonging to the class *Mollicutes*. *M. genitalium* has a cell membrane but no cell wall and lives on and in the epithelial cells of the urinary and genital tracts of men and women.

In lower risk populations, *M. genitalium* prevalence of approximately 1% to 3% has been reported in both men and women (1, 2, 3, 4). In higher risk populations, prevalence of 10% to 41% in men and 7.3% to 14% in women has been reported (3, 5, 6, 7). The prevalence of *M. genitalium* in higher risk populations often exceeds that of *Neisseria gonorrhoeae* and is similar to the prevalence of *Chlamydia trachomatis* (8, 9, 10, 11, 12).

In a review of published studies, infection with *M. genitalium* was shown to be strongly associated with non-gonoccocal urethritis (NGU) in men (13). In those subjects evaluated, *M. genitalium* was detected in 15% to 25% of men with symptomatic NGU and >30% of men with non-chlamydial NGU. In women, several studies have reported *M. genitalium* to be associated with cervicitis ($P \leq .03$; 8, 12, 14). A recent meta-analysis also shows that infection with *M. genitalium* was associated with an approximately two-fold increase in risk for cervicitis, pelvic inflammatory disease, preterm birth, spontaneous abortion, and infertility (15).

M. genitalium infections largely go unrecognized, and infected individuals are either asymptomatic or have symptoms similar to those associated with other bacterial infections of the urogenital tract. In an evaluation of men attending an STI clinic in Sweden, 61% (17/28) of men with *M. genitalium* infections were symptomatic; 93% (26/28) had signs of urethritis (14). In women, *M. genitalium* infection is often asymptomatic. In an evaluation of women attending an STI clinic in Sweden, 77% (17/22) of women with *M. genitalium* infections were asymptomatic, though many exhibited clinical signs of infection; 50% (11/22) had signs of urethritis and/or cervicitis: 2 had signs of urethritis only, 6 had signs of cervicitis only, and 3 had signs of urethritis and cervicitis (16).

In patients with relevant signs or symptoms, current treatment recommendations are focused on chlamydial, gonorrheal or trichomonal infections. However, optimal antimicrobial therapy for bacterial-associated urethritis and cervicitis is organism-specific, and therapeutic regimens effective against these organisms lack efficacy for curing *M. genitalium* infections.

Because *M. genitalium* is fastidious and difficult to culture, the United States Centers for Disease Control and Prevention recommends the use of NAATs for detecting *M. genitalium* (17). The Aptima Mycoplasma genitalium assay is a NAAT that utilizes target capture,

transcription-mediated amplification (TMA), and hybridization protection assay (HPA) technologies to detect 16s rRNA of *M. genitalium*.

Principles of the Procedure

The Aptima Mycoplasma genitalium assay involves three main steps, which all take place in a single tube on the Panther system: target capture, transcription-mediated amplification (TMA), and hybridization protection assay (HPA). The assay incorporates an internal control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.

A specimen is collected and transferred into the appropriate specimen transport tube. The transport solution in the transport tube releases the rRNA target and protects it from degradation during storage. When the Aptima Mycoplasma genitalium assay is performed in the laboratory, the target rRNA, if present, is isolated by the use of a specific capture oligomer and magnetic microparticles in a method called target capture. The capture oligomer contains a sequence complementary to a specific region of the target molecule as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific region of the capture oligomer binds to a specific region of the target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecule bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors. After the target capture steps are completed, the rRNA is 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 *M. genitalium* via DNA and RNA intermediates and generates RNA amplicon molecules. Detection of the rRNA amplification product sequences is achieved using nucleic acid hybridization. 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. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther System Operator's Manual* prior to performing this assay.

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

D. **Warning: Irritants and Corrosives:** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash with water. If these fluids spill, dilute the spill with water before wiping dry. Please reference the appropriate Safety Data Sheet for additional information.

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

Laboratory Related

F. Use only supplied or specified disposable laboratory ware.

G. Use routine laboratory precautions. Do not pipette by mouth. 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.

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

H. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Thoroughly clean and disinfect all work surfaces.

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

J. Good standard practices for molecular laboratories include environmental monitoring. To monitor a laboratory's environment, the following procedure is suggested:

   a. For each area to be tested, obtain an Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens.

   b. Label each tube appropriately.

   c. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging.

   d. To collect the surface samples, lightly moisten the specimen collection swab with nuclease-free water.

   e. Swab the surface of interest using a top to bottom vertical motion. Rotate the swab approximately one-half turn while swabbing the location.

   f. Immediately place the swab sample into the transport tube.

g. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.

h. Recap the swab transport tube tightly.

i. Repeat steps for any remaining swab samples.

j. Test swab(s) with the molecular assay.

Specimen Related

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

L. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established in accordance with applicable national, international, and regional regulations.

M. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

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

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

P. 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.

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

Assay Related

R. Do not use the reagent or calibrator kits after the expiration date.

S. 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.

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

U. Avoid microbial and ribonuclease contamination of reagents.

V. Do not interchange, mix, or combine reagents from assay kits with different lot numbers. Calibrators are not lot specific and assay fluids can be from different lot numbers.

Reagent Storage and Handling Requirements

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

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

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2ºC to 8ºC		
Enzyme Reagent	2ºC to 8ºC		
Probe Reagent	2ºC to 8ºC		
Internal Control Reagent	2ºC to 8ºC		
Amplification Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days
Enzyme Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days
Probe Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days
Target Capture Reagent	15ºC to 30ºC	15ºC to 30ºC	30 days
Selection Reagent	2ºC to 30ºC	2ºC to 30ºC	30 days
Negative Calibrator	2ºC to 8ºC		Single use vial
Positive Calibrator	2ºC to 8ºC		Single use vial

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

C. Discard any unused reconstituted reagents and working Target Capture Reagent (wTCR) after 30 days, or after the Master Lot expiration date, whichever comes first.

D. Unopened calibrators are stable until the date indicated on the vials.

E. Reconstituted reagents stored onboard the Panther system have 156 hours of onboard stability. The Panther system logs each time the reagents are loaded.

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

G. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Protect these reagents from light during storage.

H. Do not freeze reagents.

Specimen Collection and Storage

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

Clinician-collected and self-collected vaginal swab specimens, clinician-collected endocervical swab specimens, clinician-collected cervical specimens collected in PreservCyt™ solution, self-collected first-catch male and female urine specimens, clinician-collected male urethral swab specimens, and self-collected penile meatal swab specimens can be tested with the Aptima Mycoplasma genitalium assay. Assay performance has not been evaluated with specimens other than those collected with the following specimen collection kits:

- Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- Aptima Urine Collection Kit for Male and Female Urine Specimens
- Aptima Vaginal Swab Specimen Collection Kit
- Aptima Multitest Swab Specimen Collection Kit for vaginal swab specimens and penile meatal swab specimens
- Aptima Specimen Transfer Kit (for use with gynecological samples collected in PreservCyt solution)

A. Specimen collection

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

B. Specimen transport and storage before testing:

1. Swab specimens
   a. After collection, swab specimens in transport tubes can be stored at 2ºC to 30ºC for up to 60 days.
   b. If longer storage is needed, swab specimens in transport tubes can be stored at -20°C or -70°C for up to an additional 90 days.
2. Urine specimens
   a. Before urine specimens can be tested, urine must be transferred to an Aptima urine transport tube in accordance with the instructions in the urine collection kit package insert.
   b. After collection, urine specimens in the primary collection container can be stored at 2ºC to 30ºC for up to 24 hours before urine is transferred to the transport tube.
   c. Processed urine in the transport tube can be stored at 2ºC to 30ºC for up to 30 days (after transfer).
   d. If longer storage is needed, processed urine in the transport tube can be stored at -20°C or -70°C for up to an additional 90 days (after transfer).
3. Specimens collected in PreservCyt solution
   a. Before gynecologic specimens in PreservCyt solution can be tested, volume must be transferred to an Aptima specimen transfer tube in accordance with the instructions in the Aptima Specimen Transfer kit package insert.

b. After collection, gynecologic specimens in the PreservCyt solution vial can be stored at 2ºC to 30ºC for up to 30 days before volume is transferred to the Aptima specimen transfer tube.

c. Processed gynecologic specimens in the transfer tubes can be stored at 2ºC to 30ºC for up to 60 days (after transfer).

d. If longer storage is needed, processed gynecologic specimens in the transfer tube can be stored at -20°C or -70°C for up to an additional 90 days (after transfer).

C. Specimen storage after testing:

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen transport tubes should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be shipped, remove penetrable cap and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 RCF (Relative Centrifugal Force) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination.**

Specimen Transport

Maintain sample storage conditions as described in *Specimen Collection and Storage* section.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima Mycoplasma genitalium assay are listed below for the Panther system. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials

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

Aptima Mycoplasma genitalium Assay Kit

100 tests (2 boxes) (Cat. No. PRD-03374)*

100 tests (2 boxes and 1 calibrators kit) (Cat. No. PRD-03919)

Aptima Mycoplasma genitalium Refrigerated Box (store at 2ºC to 8ºC upon receipt)

Symbol	Component	Quantity
A	**Aptima Mycoplasma genitalium Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Aptima Mycoplasma genitalium Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Aptima Mycoplasma genitalium Probe Reagent** *Chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**Aptima Mycoplasma genitalium Internal Control** *Non-infectious RNA transcript in buffered solution containing < 5% detergent.*	1 vial

Aptima Mycoplasma genitalium Room Temperature Box (store at 15ºC to 30ºC upon receipt)

Symbol	Component	Quantity
AR	**Aptima Mycoplasma genitalium Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 bottle
ER	**Aptima Mycoplasma genitalium Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 bottle
PR	**Aptima Mycoplasma genitalium Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 bottle

* Calibrator kits are sold separately. See individual catalog box number below.

Aptima Mycoplasma genitalium Room Temperature Box (store at 15ºC to 30ºC upon receipt) *(continued)*

Symbol	Component	Quantity
S	**Aptima Mycoplasma genitalium Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 bottle
TCR	**Aptima Mycoplasma genitalium Target Capture Reagent** *Buffered solution containing capture oligomers and magnetic particles.*	1 bottle
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Aptima Mycoplasma genitalium Calibrators Kit (PRD-03393) (store at 2ºC to 8ºC upon receipt)

Symbol	Component	Quantity
NCAL	**Aptima Mycoplasma genitalium Negative Calibrator** *Buffered solution containing < 5% detergent.*	5 vials
PCAL	**Aptima Mycoplasma genitalium Positive Calibrator** *Non-infectious Mycoplasma genitalium in vitro RNA transcript in buffered solution containing < 5% detergent.*	5 vials

Materials Required But Available Separately

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

	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit *contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Aptima Mycoplasma genitalium Calibrators Kit	PRD-03393
Aptima Specimen Transfer Kit *for use with specimens in PreservCyt™ solution*	301154C
Aptima Vaginal Swab Specimen Collection Kit	301162

Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Aptima Urine Specimen Collection Kit	301040
Or Aptima Urine Specimen Transport Tubes	105575
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable powderless gloves	—
Aptima penetrable caps	105668
Reagent replacement Caps for 100-test kits	—
Amplification, Enzyme, and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
TCR and Selection reagent	*501604 (100 caps)*
Plastic-backed laboratory bench covers	—
Centrifuge	—

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Replacement non-penetrable caps	103036A

Panther System Test Procedure

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

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface 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).

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reconstitution of reagents should be performed prior to beginning any work on the Panther system.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the lyophilized reagent with the appropriate reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Remove the lyophilized reagents (2ºC to 8ºC) and corresponding reconstitution solutions (15ºC to 30ºC) from storage.
   b. Before attaching the reconstitution collar, ensure that the reconstitution solution and lyophilized reagent have matching label colors.
   c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   d. Open the lyophilized reagent vial by removing the metallic seal and rubber stopper. Firmly insert the notched end of the reconstitution collar (black) into the vial (Figure 1, Step 1).
   e. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
   f. Place the reconstitution solution bottle on a stable surface (i.e., bench). Then invert the lyophilized reagent vial over the reconstitution solution bottle and firmly attach the collar to the reconstitution solution bottle (Figure 1, Step 2).
   g. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial (Figure 1, Step 3).
   h. Pick up the assembled bottles and gently swirl. Avoid creating foam while swirling the bottle (Figure 1, Step 4).
   i. Wait for the lyophilized reagent to go into solution. After the lyophilized reagent has gone into solution, gently swirl to mix, then invert the assembled bottles again, tilting at a 45º angle to minimize foaming (Figure 1, Step 5). Slowly tilt the assembled bottles again to allow all of the solution to drain back into reconstitution solution bottle.
   j. Carefully remove the reconstitution collar and glass vial (Figure 1, Step 6).
   k. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

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

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

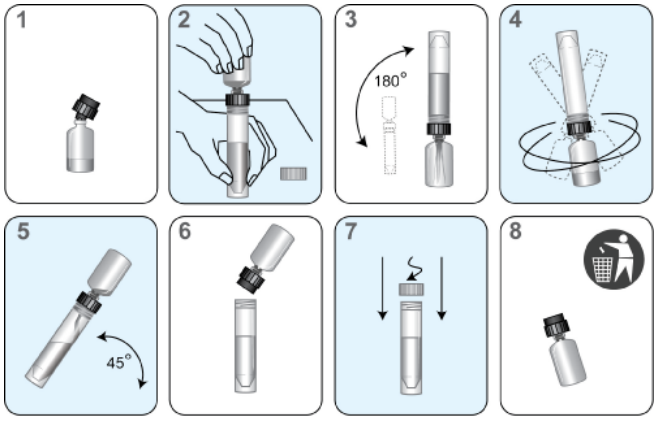

Figure 1. Reagent Reconstitution Process

2. To prepare wTCR, perform the following:
   a. Remove the appropriate bottles of TCR (15ºC to 30ºC) and Internal Control Reagent (2ºC to 8ºC) from storage.
   b. Check the lot number on the TCR bottle and Internal Control Reagent bottle to make sure that the numbers match the lot number on the Master Lot Barcode Sheet.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the bottle of Internal Control Reagent and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the Internal Control 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 Internal Control Reagent bottle and cap.
3. Prepare Selection Reagent
   a. Remove the Selection Reagent from storage (2ºC to 30ºC). Check the lot number on the Selection Reagent bottle to make sure the lot number matches the number on the Master Lot Barcode sheet.
   b. If the Selection Reagent is stored refrigerated let it come to room temperature before placing on the Panther system.
   c. 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 Prepared Reagents

1. Remove the previously prepared reagents from storage (2ºC to 8ºC). Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15ºC to 30ºC) prior to the start of the assay.

2. If reconstituted Probe Reagent contains precipitate at room temperature (15ºC to 30ºC), heat the capped bottle at a temperature that does not exceed 62ºC 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. Avoid creating foam during inversion of reagents.
3. Invert the Amplification, Enzyme, and Probe Reagents to mix thoroughly prior to loading on the system. Avoid creating excessive foam during inversion of reagents.
4. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

D. Calibrator Preparation

Remove the calibrators from storage (2ºC to 8ºC) and allow the calibrators to reach 15ºC to 30ºC prior to processing.

E. Specimen Handling

1. Allow the specimens to reach 15ºC to 30ºC prior to processing.
2. **Do not vortex specimens.**
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single blue Aptima collection swab in a unisex swab specimen transport tube.
   b. The presence of a single pink Aptima collection swab in a Multitest or Vaginal swab specimen transport tube.
   c. A final volume of urine between the black fill lines of a urine specimen transport tube.
   d. The absence of a swab or collection device in the Aptima specimen transport tube for PreservCyt™ solution specimens.
   e. If the specimen does not meet the criteria, the specimen must be rejected.
4. Inspect specimen tubes before loading into the Sample 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 a urine specimen tube contains precipitate, heat the specimen at 37ºC for up to 5 minutes.

Note: *Failure to follow Steps 4a-4c may result in liquid discharge from the specimen tube cap.*

Note: *Up to 3 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 3 aliquots from the specimen tube can lead to processing errors.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther System Operator's Manual* and *Procedural Notes*.
2. Load samples in the Sample Rack.
3. When all samples are loaded, secure the Sample Retainer onto the Sample Rack, and load the samples into the Sample Bay.

4. Repeat steps 2 to 3 for the next Sample Rack.

Procedural Notes

A. Calibrators

1. The Aptima Positive Calibrator for *Mycoplasma genitalium* and Aptima Negative Calibrator for *Mycoplasma genitalium* tubes can be loaded in any rack position or in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of calibrators is currently being processed by the system.
   b. Valid results for the calibrators are registered on the system.
2. Once the calibrator tubes have been pipetted and are processing for the Aptima Mycoplasma genitalium assay reagent kit, specimens can be tested with the associated reconstituted kit for up to 48 hours **unless:**
   a. Calibrator results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each calibrator tube can be used once. Attempts to use the tube more than once can lead to processing errors.

Quality Control

A run or specimen result may be invalidated by an operator if technical, operator, or instrument difficulties are observed while performing the assay and are documented.

All results invalidated by the instrument or operator must be retested.

Assay Calibration

To generate valid results, an assay calibration must be completed. A positive and negative calibrator are run in duplicate each time a reagent kit is loaded on the Panther system. The Panther manual lists 24 hour calibrator stability, however, the Aptima Mycoplasma genitalium assay calibration is valid for up to 48 hours. Software on the Panther system alerts the operator when a new calibrator set is required.

During processing, criteria for acceptance of the calibrator are automatically verified by the software on the Panther system. If two replicates are invalid for either the positive or negative calibrator, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared set of calibrators.

Note: *For assistance with calibrators with out-of-range error flags, contact Hologic Technical Support.*

Internal Control

Each sample contains an internal control (IC). During processing, IC acceptance criteria are automatically verified by the Panther system software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested.

The Panther system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther System Operator's Manual.*

Interpretation of Results

Assay test results are automatically interpreted by the Panther system Aptima Mycoplasma Genitalium Assay software. A test result may be negative, positive, or invalid as determined by the Internal Control (IC) Relative Light Unit (RLU) and Signal to Cutoff (S/CO) ratio for the Analyte in the detection step (see below). A test result may be invalid due to RLU values outside the normal expected ranges. Initial invalid test results should be retested. Report the first valid result.

Table 1: Result Interpretation

Assay Result	Criteria
Negative	Analyte S/CO < 1.0 IC ≥ IC Cutoff IC ≤ 1,200,000 RLU
Positive	Analyte S/CO ≥ 1.0 IC ≤ 1,200,000 RLU Analyte ≤ 3,000,000 RLU
Invalid	Analyte S/CO < 1.0 and IC < IC Cutoff Or IC > 1,200,000 RLU Or Analyte > 3,000,000 RLU

Quality Control Results and Acceptability

Run Validity Criteria

The software automatically determines run validity. The software will invalidate a run if any of the following conditions occur:

- Both Negative Calibrator replicates are invalid.
- Both Positive Calibrator replicates are invalid.

A run may be invalidated by an operator if technical, operator, or instrument difficulties are observed and documented while performing the assay.

An invalid run must be repeated. Aborted runs must be repeated.

Calibrator Acceptance Criteria

The Aptima Mycoplasma genitalium Calibrators must produce the following test results:

Table 2: Acceptance Criteria

Calibrator	RLU	*M. genitalium* Result
Negative Calibrator Analyte	≥ 0 and ≤ 40,000	Valid
Negative Calibrator IC	≥ 120,000 and ≤ 425,000	Valid
Positive Calibrator Analyte	≥ 650,000 and ≤ 2,700,000	Valid
Positive Calibrator IC	≥ 0 and ≤ 800,000	Valid

IC Cutoff Calculation

The IC cutoff is determined from the IC signal from valid Negative Calibrator replicates.

IC Cutoff = 0.5 x [mean IC RLU of the valid Negative Calibrator replicates]

Analyte Cutoff Calculation

The analyte cutoff is determined from the RLU signal from valid Negative Calibrator replicates and valid Positive Calibrator replicates.

*Analyte Cutoff = [1 * mean analyte RLU of valid Negative Calibrator replicates] + [0.035 x mean analyte RLU of the valid Positive Calibrator replicates]*

Analyte Signal to Cutoff (S/CO) Calculation

The analyte S/CO is determined from the analyte RLU of the test sample and the analyte cutoff for the run.

Analyte S/CO = test sample analyte RLU ÷ analyte cutoff

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 evaluated for their impact on the detection of *M. genitalium*.

C. Urine, vaginal swab, and PreservCyt™ solution 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.

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

E. Reliable results are dependent on adequate specimen collection, transport, storage, and processing. 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.

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

G. Results from the Aptima Mycoplasma genitalium assay should be interpreted in conjunction with other clinical data available to the clinician.

H. 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.

I. The Aptima Mycoplasma genitalium 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.

J. Performance using any female specimen types has not been determined in pregnant women.

K. Performance of the assay has not been evaluated in women less than 19 years of age.

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

M. Customers must independently validate an LIS transfer process.

N. The performance of gynecological specimens collected in the PreservCyt solution vial and processed with the ThinPrep™ systems has not been established for the Aptima Mycoplasma genitalium assay.

Panther System Assay Performance

Performance in Clinical Specimens and Contrived Positive Samples

The performance of the Aptima Mycoplasma genitalium assay was compared to an alternate target *M. genitalium* TMA assay. A total of 1,422 samples were collected from subjects in Europe, Canada, and the United States using Aptima specimen collection kits. Clinician and self-collected vaginal swabs (n=173), endocervical swabs (n=177), PreservCyt™ liquid based cytology samples (n= 352), female urine (n=302), male urine (n=133), male urethral swabs (n=136), and self-collected penile meatal swabs (n=149) were tested with both assays in house. In addition, contrived clinical samples of each sample type, except male urine, spiked with *M. genitalium* whole cell lysate were included in the study. The *M. genitalium* concentration of the spiked samples was 0.1 CFU/mL (0.025 CFU/reaction), representing a half log below the lowest possible concentration of *M. genitalium* in a clinical sample. The positive and negative agreements were calculated for each sample type, combining clinical specimens and contrived samples, and are presented in Table 3.

Table 3: Positive and negative agreement of the Aptima Mycoplasma genitalium assay (AMG) compared to an alternate target M. genitalium TMA assay (ALT TMA)

Specimen Type	N	AMG +	AMG +	AMG -	AMG -	Positive Agreement	Negative Agreement	Overall Agreement
		ALT TMA +	ALT TMA -	ALT TMA +	ALT TMA -	(95% CI)	(95% CI)	(95% CI)
Penile Meatal Swab	149*	64	2	0	83	100.0% (94.3-100%)	97.6% (91.4-99.4%)	98.7% (95.2-99.6%)
Male Urine	133	45	1	0	87	100.0% (92.1-100%)	98.9% (93.8-99.8%)	99.2% (95.9-99.9%)
Male Urethral Swab	136*	39	0	0	97	100.0% (91.0-100%)	100% (96.2-100%)	100% (97.3-100%)
Female Urine	302*	59	0	0	243	100.0% (93.9-100%)	100.0% (98.4-100%)	100% (98.7-100%)
PreservCyt Liquid Based Cytology Sample	352*	59	1	0	292	100.0% (93.9-100%)	99.7% (98.1-99.9%)	99.7% (98.4-100%)
Vaginal Swab	173*	69	2	0	102	100.0% (94.7-100%)	98.1% (93.3-99.5%)	98.8% (95.9-99.7%)
Endocervical Swab	177*	64	0	0	113	100% (94.3-100%)	100% (96.7-100%)	100% (97.9-100%)

* Number of spiked samples: Penile Meatal swabs = 49; Urethral Swabs = 25; Female Urine Specimens = 49; PreservCyt Samples = 52; Vaginal Swabs = 46; Endocervical Swabs = 50.

Assay Reproducibility

Aptima Mycoplasma genitalium assay reproducibility was evaluated using the Panther system. Testing was performed over three days using two lots of assay reagents and three operators using three Panther systems. Reproducibility panels were created by spiking specimen transport media (STM) with the appropriate amount of *M. genitalium* RNA transcript. Final *M. genitalium* RNA concentrations were 0 and 100 copies/mL. Table 4 presents, for each panel member, S/CO data in terms of mean, standard deviation (SD), and coefficient of variation (CV) between operators, between instruments, between days, between lots, between runs, within runs, and overall (total). Table 5 shows the positivity and the percent agreement of the panels. There were no false negatives and one false positive in the study. Samples with valid results were included in the analyses.

Table 4: Reproducibility Study: Reproducibility of the Aptima Mycoplasma genitalium Assay by Panel

Panel	N	Mean S/CO	Between Operators		Between Instruments		Between Days		Between Lots		Between Runs		Within Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative Panel	1,438	0.01	0	0	0	0	0	0	0.00	31.13	0	0	0.36	3,699	0.36	3,699
Positive Panel	1,434	25.73	0.22	0.85	0.30	1.16	0	0	0.12	0.45	0.80	3.11	1.23	4.79	1.52	5.90

N = number; SD = standard deviation; CV = coefficient of variation; S/CO = signal to cutoff ratio.

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD=0 and CV=0%.

Table 5: Aptima Mycoplasma genitalium Assay Panel Description Percent Agreement

Description	Valid N	% Positive	% Agreement
Negative Panel	1,438	0.07% (0.01-0.39)	99.93% (99.61-99.99)
Positive Panel	1,434	100% (99.73-100)	100% (99.73-100)

Analytical Sensitivity

Sensitivity panels containing 0.01 CFU/mL in STM were prepared with *M. genitalium* lysate. Testing showed 100% positivity at 0.01 CFU/mL.

Cross-Reactivity in the Presence of Microorganisms

Specificity

Specificity of the Aptima Mycoplasma genitalium 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 with 20 replicates of each isolate. The list of organisms and the concentrations tested are provided in Table 6. No cross-reactivity in the Aptima Mycoplasma genitalium assay was observed with any of the organisms tested.

Sensitivity

Sensitivity of the Aptima Mycoplasma genitalium assay was evaluated by testing the same organisms (Table 6) in STM spiked with *M. genitalium* lysate to a final concentration of 0.25 CFU/mL (20 replicates of each isolate). No interference was observed in the presence of the microorganisms tested.

Table 6: Microorganisms Tested in the Aptima Mycoplasma genitalium Assay on the Panther System

Microorganism	Concentration	Microorganism	Concentration
Acinetobacter lwoffii	$1x10^6$ CFU/mL	Human papillomavirus type 16 (SiHa cells)	$1x10^4$ cells/mL
Actinomyces israelii	$1x10^6$ CFU/mL	*Klebsiella pneumoniae*	$1x10^6$ CFU/mL
Alcaligenes faecalis	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^6$ CFU/mL
Atopobium vaginae	$1x10^6$ CFU/mL	*Lactobacillus crispatus*	$1x10^6$ CFU/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Leptotrichia buccalis*	$1x10^6$ CFU/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Campylobacter jejuni	$1x10^6$ CFU/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Candida albicans	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^6$ CFU/mL
Chlamydia trachomatis	$1x10^6$ CFU/mL	*Mycoplasma pneumoniae*	250 CFU/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Peptostreptococcus magnus*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	*Prevotella bivia*	$1x10^6$ CFU/mL
Cytomegalovirus	$1x10^5$ TCID 50/mL	*Propionibacterium acnes*	$1x10^6$ cells/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Escherichia coli	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Fusobacterium nucleatum	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Gardnerella vaginalis	$1x10^6$ CFU/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
Herpes simplex virus type 1	$2.5 x10^6$ TCID 50/mL	*Trichomonas vaginalis*	$1x10^6$ CFU/mL
Herpes simplex virus type 2	$2.5 x10^6$ TCID 50/mL	*Ureaplasma parvum*	$1x10^6$ CFU/mL
HIV-1	$1x10^6$ copies/mL	*Ureaplasma urealyticum*	$1x10^6$ cells/mL

Interference

Endogenous and exogenous substances were individually spiked into STM to a final concentration of 1% (vol/vol or wt/vol) for personal lubricants, personal deodorants, spermicides, and antifungals, 0.3% for porcine gastric mucus, and 5% for whole blood.

To test the effects of urine metabolites KOVA-Trol I High Abnormal w/ Urobilinogen Urinalysis Control was diluted into urine transport medium (UTM) in place of urine. This human urine-based urinalysis control material contains potential interferents such as protein (albumin), bilirubin, glucose, ketones, red blood cells, nitrite, urobilinogen and leukocytes. Glacial acetic acid was tested by spiking into PreservCyt™-STM (1% final concentration).

No interference was observed with any of the substances when spiked with *M. genitalium* whole cell lysate to a final concentration of 0.25 CFU/mL and tested in the Aptima Mycoplasma genitalium assay.

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