Aptima® Mycoplasma genitalium Assay

For *in vitro* diagnostic use.

Rx only.

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 suspected of *M. genitalium* infection.

The assay may be used to test the following specimens: clinician-collected and self-collected vaginal swabs (in a clinical setting), clinician-collected endocervical swabs, female and male urine, clinician-collected male urethral swabs, and self-collected penile meatal swabs (in a clinical setting).

For females, a vaginal swab is the preferred specimen type due to higher clinical sensitivity for detecting *M. genitalium* than other specimen types; however, female urine or clinician-collected endocervical swabs may be used as alternative specimens when vaginal swab specimens are not available. If female urine or clinician-collected endocervical swab specimens test negative, testing with a vaginal swab may be indicated, if *M. genitalium* infection is suspected.

Summary and Explanation of the Test

M. genitalium is a sexually-transmitted 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). In higher risk populations, prevalence of 9% to 24% in men and 11% to 16% in women has been reported (4, 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* (6, 7, 8, 9, 10, 11, 12, 13, 14).

In a review of published studies, infection with *M. genitalium* was shown to be strongly associated with non-gonococcal urethritis (NGU) in men (9, 15). In evaluated subjects, *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 (8, 12, 16). 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 (17).

M. genitalium infections largely go unrecognized, as 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 (16). 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 (18).

In patients with relevant signs or symptoms, current treatment recommendations are focused on chlamydial, gonorrheal or trichomonal infections. However, antimicrobial therapy for these bacterial- or protozoal-associated urethritis and cervicitis is organism-specific, and therapeutic

regimens effective against these organisms have reduced efficacy for curing *M. genitalium* infections.

Because *M. genitalium* is fastidious and difficult to culture, the United States Centers for Disease Control and Prevention and the Public Health Agency of Canada recommend the use of NAATs for detecting *M. genitalium* (19, 20). 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 3 main steps, which all take place in a single tube on the Panther System: target capture, TMA, and 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 RNA from *M. genitalium* via DNA and RNA intermediates and generates RNA amplicon molecules. Detection of the RNA amplicon sequences is achieved using nucleic acid hybridization. A single-stranded chemiluminescent DNA probe, which is complementary to a region of the RNA amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with RNA amplicons to form stable DNA:RNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, photons emitted from the labeled DNA:RNA hybrids are measured in a luminometer and are reported as relative light units (RLU). Final assay results are interpreted based on the analyte signal-to-cutoff (S/CO).

Warnings, Precautions, and Other Limiting Statements

A. For *in vitro* diagnostic use.

B. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther/Panther® Fusion System Operator's Manual* prior to performing 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: Irritant and Corrosive:** Avoid contact of Auto Detect 2 with skin, eyes, and mucous membranes. If this fluid comes into contact with skin or eyes, wash with water. If this fluid spills, dilute the spill with water before wiping dry.

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

Laboratory Related

F. Use only supplied or specified disposable laboratory ware.

G. Use routine laboratory precautions. Do not 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% (0.35 M to 0.5 M) 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. 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

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. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. 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 2 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, 2 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.

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.

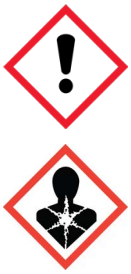
U. Avoid microbial and ribonuclease contamination of reagents.

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.

W. Some reagents in this kit are labeled with hazard information.

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

US Hazard Information



Selection Reagent
BORIC ACID 1 – 5%
TRITON X-100 1 – 5%
DANGER
H315 – Causes skin irritation.
H360FD - May damage fertility. May damage the unborn child.
P264 – Wash face, hands and any exposed skin thoroughly after handling.
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of water and soap.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P362 + P364 Take off contaminated clothing and wash before reuse.
P201 - Obtain special instructions before use.
P202 - Do not handle until all safety precautions have been read and understood.
P308 + P313 - IF exposed or concerned: Get medical advice/attention.
P405 - Store locked up.
P501 - Dispose of contents/container to an approved waste disposal plant

Limiting Statements

A. A negative result does not preclude a possible infection. Test results may be affected by improper specimen collection, technical error, specimen mix-up, or target levels below the assay limit of detection (LoD).

B. Results from the Aptima Mycoplasma genitalium Assay should be interpreted in conjunction with other clinical and laboratory data available to the clinician.

C. Reliable results are dependent on adequate specimen collection, transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results. 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.

D. For females, a vaginal swab is the preferred specimen type due to higher clinical sensitivity for detecting *M. genitalium* than other specimen types; however, female urine or clinician-collected endocervical swabs may be used as alternative specimens when vaginal swab specimens are not available. If female urine or clinician-collected endocervical swab specimens test negative, testing with a vaginal swab may be indicated, if *M. genitalium* infection is suspected.

Reagent Storage and Handling Requirements

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

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

D. Controls 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 (in a clinical setting), clinician-collected endocervical swab specimens, female and male urine specimens, clinician-collected male urethral swab specimens, and self-collected penile meatal swab specimens (in a clinical setting) 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® Multitest Swab Specimen Collection Kit

A. Specimen collection

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

B. Specimen 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).

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 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 previously tested and recapped samples, 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

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

* Calibrators 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
Panther Fusion System	PRD-04172
Panther System Continuous Fluids and Waste (Panther Plus)	PRD-06067
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, filtered, conductive, liquid sensing, and disposable *Not all products are available in all regions. Contact your representative for region-specific information.*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima® Mycoplasma genitalium Calibrators Kit	PRD-03393

	Cat. No.
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 8.25% (0.7 M to 1.16 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
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 (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).
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.
   a. Allow the lyophilized reagents to reach room temperature (15ºC to 30ºC) 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 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. 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). 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 label cap that corresponds to the reagent or a 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).

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

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

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

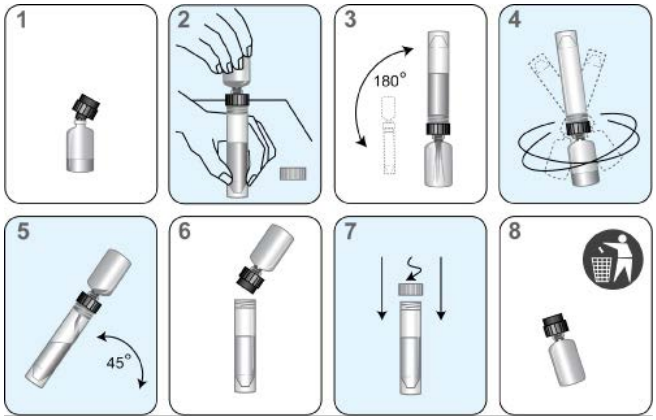


Figure 1. Reagent Reconstitution Process

2. To prepare wTCR, perform the following:
   a. Pair the appropriate bottles of TCR and IC.
   b. Check the lot number on the TCR bottle and IC 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 IC Reagent and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
   e. Cap the bottle 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 IC Reagent bottle and cap.
3. Prepare Selection Reagent
   a. 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. 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. Previously prepared Amplification, Enzyme and Probe Reagents must reach room temperature (15ºC to 30ºC) 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 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, 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. 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. Visually confirm that each specimen tube meets 1 of the following criteria:
   a. The presence of a single blue collection swab in a unisex swab specimen transport tube.
   b. The presence of a single pink 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. If the specimen does not meet the criteria, the specimen must be rejected.
2. Allow the specimens to reach 15ºC to 30ºC 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 in the tube).*

3. 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 3a-3c 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/Panther Fusion System Operator's Manual* and *Procedural Notes*.

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

2. Load samples.

Procedural Notes

A. Calibrators

1. The Aptima Positive Calibrator for *Mycoplasma genitalium* tube and the Aptima Negative Calibrator for *Mycoplasma genitalium* tube can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Specimen pipetting will begin when 1 of the following 2 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.

B. 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 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 Aptima Mycoplasma genitalium Assay on the Panther System.

8. Further investigation should be performed if any samples yield a positive result.

For test interpretation, see Test Interpretation- QC/Patient Results. For additional Panther System-specific contamination monitoring information, contact Hologic Technical Support.

Quality Control and Calibration

Assay Calibration

To generate valid results, an assay calibration must be completed. One (1) positive calibrator tube and 1 negative calibrator tube are run in duplicate each time a reagent kit is loaded on the Panther System. 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 2 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.*

Controls

Each sample contains an internal control. 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/Panther Fusion System Operator's Manual.*

Note: *External assay performance control samples (not provided) should be tested in conformance with local, state, and/or federal regulations or accreditation requirements and each laboratory's standard Quality Control procedures.*

Interpretation of Results

Assay test results are automatically interpreted by the assay software. A test result may be negative, positive, or invalid as determined by the 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. The first valid result is the result that should be reported. Samples with invalid test results should retested. If the result is invalid upon retest, a new specimen should be collected.

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 x mean analyte RLU of valid Negative Calibrator replicates] + [0.035 x mean analyte RLU of the valid Positive Calibrator replicates]

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

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

E. Interference in assay results was observed when mucus at a final concentration of 0.3% w/v was added to clinical specimen matrix. Interference was not observed when mucus at a final concentration of 0.03% w/v was added to clinical specimen matrix.

F. 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.

G. Performance of the assay has not been evaluated in individuals less than 15 years of age.

H. If a urine 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.

I. Customers must independently validate an LIS transfer process.

J. In rare cases, specimens collected from patients with urogenital tract co-infections with low *M. genitalium* titer (approximately 5 *M. genitalium* organisms/swab) and high *M. pneumoniae* titer (1×10^5 CFU/mL) may result in a false-negative result using the Aptima Mycoplasma genitalium Assay. Lower or higher titers of *M. pneumoniae* in the presence of low titer *M. genitalium* may result in a reduced positive assay signal or an invalid assay result, respectively.

Panther System Expected Values

Prevalence

The prevalence of *M. genitalium* in patient populations depends on risk factors such as age, gender, the presence or absence of symptoms, the type of clinic, and the sensitivity of the test used to detect infections. A summary of the positivity of *M. genitalium* rRNA detection, as determined by the Aptima Mycoplasma genitalium Assay on the Panther System, is shown in Table 3 for the multi-center study, by clinical site and overall.

Table 3: Positivity of M. genitalium as Determined by the Aptima Mycoplasma genitalium Assay by Specimen Type and Clinical Site

	% Positivity (# positive/# tested with valid results)						
Site	**CVS**	**PVS**	**ES**	**FU**	**US**	**PM**	**MU**
1	17.1 (6/35)	20.0 (7/35)	18.2 (6/33)	17.1 (6/35)	12.6 (14/111)	12.6 (14/111)	10.8 (12/111)
2	17.6 (3/17)	17.6 (3/17)	23.5 (4/17)	11.8 (2/17)	9.1 (2/22)	13.6 (3/22)	13.6 (3/22)
3	7.1 (12/168)	7.1 (12/169)	4.7 (8/169)	5.4 (9/168)	2.6 (3/115)	1.8 (2/113)	2.6 (3/115)
4	--	--	--	--	18.5 (5/27)	22.2 (6/27)	22.2 (6/27)
5	50.0 (1/2)	50.0 (1/2)	50.0 (1/2)	0.0 (0/2)	--	--	--
6	10.3 (3/29)	13.8 (4/29)	10.3 (3/29)	13.8 (4/29)	11.1 (2/18)	22.2 (4/18)	11.1 (2/18)
7	12.2 (11/90)	13.2 (12/91)	11.0 (10/91)	12.1 (11/91)	0.0 (0/17)	0.0 (0/17)	0.0 (0/17)
8	16.2 (12/74)	17.3 (13/75)	13.3 (10/75)	10.7 (8/75)	17.8 (8/45)	16.7 (7/42)	13.3 (6/45)
9	9.1 (10/110)	10.7 (12/112)	7.2 (8/111)	8.0 (9/112)	16.7 (24/144)	16.0 (23/144)	17.4 (25/144)
10	0.0 (0/30)	0.0 (0/30)	0.0 (0/30)	0.0 (0/30)	1.7 (1/59)	0.0 (0/59)	1.7 (1/59)
11	3.6 (3/83)	3.4 (3/89)	3.6 (3/83)	2.2 (2/91)	5.4 (5/93)	6.7 (6/90)	5.4 (5/93)
12	9.5 (2/21)	9.5 (2/21)	9.5 (2/21)	4.8 (1/21)	--	--	--
13	10.4 (30/288)	10.5 (30/286)	9.8 (28/287)	8.0 (23/289)	14.1 (19/135)	14.1 (19/135)	12.6 (17/135)
14	12.5 (11/88)	11.2 (10/89)	10.3 (9/87)	9.0 (8/89)	10.3 (10/97)	13.4 (13/97)	9.7 (9/93)
15	16.7 (8/48)	16.3 (8/49)	14.9 (7/47)	12.2 (6/49)	18.0 (9/50)	20.0 (10/50)	18.0 (9/50)
16	8.3 (20/242)	7.8 (19/244)	6.4 (16/249)	6.4 (16/250)	6.5 (22/340)	5.9 (20/340)	5.6 (19/340)
17	17.7 (49/277)	18.7 (52/278)	15.8 (44/278)	15.5 (43/278)	22.3 (25/112)	20.5 (23/112)	22.3 (25/112)
18	--	--	--	--	23.1 (3/13)	30.8 (4/13)	23.1 (3/13)

Table 3: Positivity of M. genitalium as Determined by the Aptima Mycoplasma genitalium Assay by Specimen Type and Clinical Site (continued)

	% Positivity (# positive/# tested with valid results)						
Site	**CVS**	**PVS**	**ES**	**FU**	**US**	**PM**	**MU**
19	12.9 (4/31)	12.5 (4/32)	10.0 (3/30)	6.5 (2/31)	17.9 (7/39)	20.5 (8/39)	15.4 (6/39)
20	0.0 (0/12)	0.0 (0/12)	0.0 (0/12)	0.0 (0/12)	5.7 (3/53)	7.5 (4/53)	3.8 (2/53)
21	7.8 (5/64)	7.8 (5/64)	7.8 (5/64)	4.7 (3/64)	8.2 (6/73)	12.5 (9/72)	6.8 (5/73)
All	11.1 (190/1709)	11.4 (197/1724)	9.7 (167/1715)	8.8 (153/1733)	10.7 (168/1563)	11.3 (175/1554)	10.1 (158/1559)

CVS = clinician-collected vaginal swab, ES = endocervical swab, FU = female urine, MU = male urine, PM = penile meatal swab, PVS = patient-collected vaginal swab, US = male urethral swab.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive and negative predictive values (PPV and NPV) of the Aptima Mycoplasma genitalium Assay for different hypothetical prevalence rates are shown for each specimen type in Table 4. For each specimen type, the PPV and NPV are derived for different hypothetical prevalence rates using the sensitivity and specificity estimates from the multi-center clinical study (see Table 5).

Table 4: Positive and Negative Predictive Values for Hypothetical Prevalence Rates by Specimen Type

Specimen Type		**Hypothetical Prevalence**						
		1%	**2%**	**5%**	**10%**	**15%**	**20%**	**25%**
CVS	PPV (%)	32.2	49.0	71.2	83.9	89.3	92.2	94.0
	NPV (%)	99.9	99.8	99.6	99.1	98.6	98.0	97.3
PVS	PPV (%)	39.2	56.6	77.1	87.6	91.8	94.1	95.5
	NPV (%)	100	100	99.9	99.9	99.8	99.7	99.6
ES	PPV (%)	32.8	49.7	71.8	84.3	89.5	92.4	94.2
	NPV (%)	99.8	99.6	99.0	98.0	96.8	95.5	94.1
FU	PPV (%)	43.3	60.7	79.9	89.4	93.0	95.0	96.2
	NPV (%)	99.8	99.5	98.8	97.6	96.2	94.7	93.1
US	PPV (%)	69.8	82.4	92.3	96.2	97.6	98.3	98.7
	NPV (%)	100	100	99.9	99.8	99.7	99.5	99.4
PM	PPV (%)	29.3	45.5	68.3	82.0	87.8	91.1	93.2
	NPV (%)	99.9	99.8	99.4	98.7	98.0	97.1	96.2
MU	PPV (%)	58.7	74.2	88.1	94.0	96.1	97.2	97.9
	NPV (%)	99.9	99.8	99.5	99.0	98.4	97.8	97.0

CVS = clinician-collected vaginal swab, ES = endocervical swab, FU = female urine, MU = male urine, NPV = negative predictive value, PM = penile meatal swab, PPV= positive predictive value, PVS = patient-collected vaginal swab, US = male urethral swab.

Panther System Clinical Performance

A prospective, multi-center clinical study was conducted to establish the clinical performance characteristics of the Aptima Mycoplasma genitalium Assay on the Panther System. Specimens were collected from 3393 symptomatic and asymptomatic men and women enrolled from 21 geographically and ethnically diverse US clinical sites, including obstetrics and gynecology, family planning, public health, and STD 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. Ninety-three enrolled subjects were not evaluable (32 subjects were withdrawn and 61 had unknown patient infected status [PIS]). Of the 3300 evaluable subjects, 1737 were women and 1563 were men; 4 were 15 to 17 years of age, 242 were 18 to 20 years of age, 483 were 21 to 24 years of age, 1954 were 25 to 44 years of age, 572 were 45 to 64 years of age, and 45 were ≥65 years of age.

Up to 3 specimens were collected from each male subject (1 urethral swab, 1 penile meatal, and 1 first-catch urine, in that order) and up to 4 specimens were collected from each female subject (1 first-catch urine, 1 patient-collected vaginal swab, 1 clinician-collected vaginal swab, and 1 endocervical swab, in that order). All specimens were clinician-collected except urine specimens, penile meatal specimens, and the patient-collected vaginal swab specimens, which were collected by the subject at the clinic.

Samples were tested with the Aptima Mycoplasma genitalium Assay on the Panther System, and with up to 3 validated reference alternate TMA assays. Samples with initial invalid Aptima Mycoplasma genitalium Assay results or instrument processing errors were retested; valid retest results were included in the performance analyses. Alternate TMA assay results from male urethral and self-collected vaginal swab samples were used to establish the PIS. Subjects were categorized as infected if a positive result occurred in at least 2 alternate TMA assays (see Table 8 and Table 9 for the PIS algorithms). Subjects that could not be categorized as infected or not infected were excluded from the PIS-based performance analyses. The alternate TMA assay results from each specimen were also used to establish specimen-specific *M. genitalium* infection status.

Of the specimens collected, 11,827 were processed in valid Aptima Mycoplasma genitalium Assay runs. Of these, 11,774 (99.6%) had final valid results and 53 (0.4%) had final invalid results and were excluded from the analyses. For the 3300 evaluable subjects, a total of 11,557 specimens were included in the analyses comparing Aptima Mycoplasma genitalium Assay results to the PIS: 1709 clinician-collected vaginal swab, 1724 patient-collected vaginal swab, 1715 endocervical swab, 1733 female urine, 1563 urethral swab, 1554 penile meatal swab, and 1559 male urine samples. The remaining 217 specimens with final valid Aptima Mycoplasma genitalium Assay results were excluded from these analyses due to unknown PIS but were included in specimen-specific agreement analyses if the specimen-specific composite reference result was available.

Performance Results

Performance characteristics of the Aptima Mycoplasma genitalium Assay were calculated for each specimen type by comparing Aptima Mycoplasma genitalium Assay results to the PIS. The sensitivity, specificity, PPV, and NPV of the Aptima Mycoplasma genitalium Assay for *M. genitalium* detection and the prevalence of *M. genitalium* (based on the infected status) are shown for all female and male specimens overall in Table 5 and by symptom status in Table 6. The positive and negative likelihood ratios (PLR, NLR) of the Aptima Mycoplasma genitalium

Assay for *M. genitalium* detection are shown for all female and male specimens overall and by symptom status in Table 7.

Table 5: Performance Characteristics in Female and Male Specimens

Specimen Type	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
CVS	1709	160	30	1505	14	10.2	92.0 (86.9-95.1)	98.0 (97.2-98.6)	84.2 (79.1-88.6)	99.1 (98.5-99.5)
PVS	1724	173	24	1525	2	10.2	98.9 (95.9-99.7)	98.5 (97.7-99.0)	87.8 (83.1-91.7)	99.9 (99.5-100)
ES	1715	141	26	1516	32	10.1	81.5 (75.1-86.6)	98.3 (97.5-98.8)	84.4 (78.9-89.1)	97.9 (97.2-98.5)
FU	1733	137	16	1541	39	10.2	77.8 (71.1-83.3)	99.0 (98.3-99.4)	89.5 (84.3-93.6)	97.5 (96.8-98.2)
US	1563	162	6	1392	3	10.6	98.2 (94.8-99.4)	99.6 (99.1-99.8)	96.4 (92.7-98.6)	99.8 (99.4-100)
PM	1554	145	30	1360	19	10.6	88.4 (82.6-92.5)	97.8 (96.9-98.5)	82.9 (77.4-87.6)	98.6 (97.9-99.1)
MU	1559	149	9	1386	15	10.5	90.9 (85.5-94.4)	99.4 (98.8-99.7)	94.3 (90.0-97.2)	98.9 (98.3-99.4)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, FU = female urine, MU = male urine, NPV = negative predictive value, PM = penile meatal swab, PPV = positive predictive value, Prev = prevalence, PVS = patient-collected vaginal swab, TN = true negative, TP = true positive, US = male urethral swab.

[1]Score CI.

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

Table 6: Performance Characteristics by Symptom Status in Female and Male Specimens

Specimen Type	Symptom Status	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
CVS	Sym	1040	112	22	898	8	11.5	93.3 (87.4-96.6)	97.6 (96.4-98.4)	83.6 (77.3-88.8)	99.1 (98.3-99.6)
	Asym	669	48	8	607	6	8.1	88.9 (77.8-94.8)	98.7 (97.5-99.3)	85.7 (75.8-92.9)	99.0 (98.0-99.6)
PVS	Sym	1047	121	18	908	0	11.6	100 (96.9-100)	98.1 (96.9-98.8)	87.1 (81.1-91.9)	100 (99.6-100)
	Asym	677	52	6	617	2	8.0	96.3 (87.5-99.0)	99.0 (97.9-99.6)	89.7 (80.4-95.7)	99.7 (98.9-100)
ES	Sym	1046	101	17	909	19	11.5	84.2 (76.6-89.6)	98.2 (97.1-98.9)	85.6 (79.1-90.8)	98.0 (97.0-98.7)
	Asym	669	40	9	607	13	7.9	75.5 (62.4-85.1)	98.5 (97.2-99.2)	81.6 (70.3-90.2)	97.9 (96.8-98.8)
FU	Sym	1051	97	15	914	25	11.6	79.5 (71.5-85.7)	98.4 (97.4-99.0)	86.6 (80.0-91.8)	97.3 (96.3-98.2)
	Asym	682	40	1	627	14	7.9	74.1 (61.1-83.9)	99.8 (99.1-100)	97.6 (88.7-99.9)	97.8 (96.7-98.7)
US	Sym	866	102	1	761	2	12.0	98.1 (93.3-99.5)	99.9 (99.3-100)	99.0 (94.9-100)	99.7 (99.1-100)
	Asym	697	60	5	631	1	8.8	98.4 (91.3-99.7)	99.2 (98.2-99.7)	92.3 (84.0-97.3)	99.8 (99.2-100)

Table 6: Performance Characteristics by Symptom Status in Female and Male Specimens (continued)

Specimen Type	Symptom Status	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
PM	Sym	865	92	17	745	11	11.9	89.3 (81.9-93.9)	97.8 (96.5-98.6)	84.4 (77.5-90.0)	98.5 (97.6-99.2)
	Asym	689	53	13	615	8	8.9	86.9 (76.2-93.2)	97.9 (96.5-98.8)	80.3 (70.8-88.1)	98.7 (97.7-99.4)
MU	Sym	866	93	7	755	11	12.0	89.4 (82.0-94.0)	99.1 (98.1-99.6)	93.0 (86.9-96.9)	98.6 (97.6-99.3)
	Asym	693	56	2	631	4	8.7	93.3 (84.1-97.4)	99.7 (98.9-99.9)	96.6 (89.0-99.5)	99.4 (98.5-99.8)

Asym = asymptomatic, CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, FU = female urine, MU = male urine, NPV = negative predictive value, PM = penile meatal swab, PPV = positive predictive value, Prev = prevalence, PVS = patient-collected vaginal swab, Sym = symptomatic, TN = true negative, TP = true positive, US = male urethral swab.

[1] Score CI.

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

Table 7: Likelihood Ratios by Symptom Status in Female and Male Specimens

Specimen Type	Symptom Status	N	PLR	NLR
CVS	Sym	1040	39.03	0.07
	Asym	669	68.33	0.11
	Overall (95% CI)[1]	1709	47.05 (33.38-68.76)	0.08 (0.05-0.13)
PVS	Sym	1047	51.44	0.00
	Asym	677	99.99	0.04
	Overall (95% CI)[1]	1724	63.80 (43.39-97.94)	0.01 (0.00-0.04)
ES	Sym	1046	45.85	0.16
	Asym	669	51.66	0.25
	Overall (95% CI)[1]	1715	48.34 (33.33-72.74)	0.19 (0.13-0.25)
FU	Sym	1051	49.24	0.21
	Asym	682	465.19	0.26
	Overall (95% CI)[1]	1733	75.75 (47.46-128.60)	0.22 (0.17-0.29)
US	Sym	866	747.35	0.02
	Asym	697	125.11	0.02
	Overall (95% CI)[1]	1563	228.76 (106.81-605.24)	0.02 (0.00-0.05)
PM	Sym	865	40.04	0.11
	Asym	689	41.97	0.13
	Overall (95% CI)[1]	1554	40.97 (29.01-59.76)	0.12 (0.07-0.18)

Table 7: Likelihood Ratios by Symptom Status in Female and Male Specimens (continued)

Specimen Type	Symptom Status	N	PLR	NLR
MU	Sym	866	97.34	0.11
	Asym	693	295.40	0.07
	Overall (95% CI)[1]	1559	140.82 (76.20-294.73)	0.09 (0.05-0.15)

Asym = asymptomatic, CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FU = female urine, MU = male urine, NLR = negative likelihood ratio, PM = penile meatal swab, PLR = positive likelihood ratio, PVS = patient-collected vaginal swab, Sym = symptomatic, US = male urethral swab.

[1] Exact 95% CI for the ratio of 2 independent proportions.

Infected Status Tables

The frequency of test outcomes from reference alternate TMA assay and investigational Aptima Mycoplasma genitalium Assay testing are summarized for female and male specimens in Table 8 and Table 9.

Table 8: Mycoplasma genitalium Patient Infected Status for Female Specimens

Patient Infected Status	Self-Collected Vaginal Swab			Aptima Mycoplasma genitalium Assay				Symptom Status	
	Alt TMA Assay #1	Alt TMA Assay #2	Alt TMA Assay #3[1]	Self-Collected Vaginal Swab	Clinician-Collected Vaginal Swab	Endocervical Swab	Urine	Sym	Asym
Infected	+	+	N/A	+	+	+	+	71	25
Infected	+	+	N/A	+	+	+	-	14	8
Infected	+	+	N/A	+	+	-	+	7	8
Infected	+	+	N/A	+	+	-	-	4	0
Infected	+	+	N/A	+	-	+	-	0	1
Infected	+	+	N/A	+	-	-	+	1	0
Infected	+	+	N/A	+	-	-	-	0	1
Infected	+	+	N/A	+	-	NR	+	1	0
Infected	+	+	N/A	+	NR	+	+	1	0
Infected	+	+	N/A	-	+	-	-	0	1
Infected	+	+	N/A	NR	NR	+	+	1	0
Infected	+	-	+	+	+	+	+	0	1
Infected	+	NR	+	+	+	+	+	1	2
Infected	-	+	+	+	+	+	+	10	2
Infected	-	+	+	+	+	+	-	2	0
Infected	-	+	+	+	+	-	+	1	0
Infected	-	+	+	+	+	-	-	1	0
Infected	-	+	+	+	+	NR	-	1	0
Infected	-	+	+	+	-	+	+	1	0
Infected	-	+	+	+	-	-	+	2	1
Infected	-	+	+	+	-	-	-	3	1
Infected	-	+	+	+	-	NR	-	0	1
Infected	-	+	+	-	-	-	-	0	1
Infected	NR	+	+	+	+	+	+	0	1
Not infected	+	-	-	+	+	+	+	1	0

Table 8: Mycoplasma genitalium Patient Infected Status for Female Specimens (continued)

Patient Infected Status	Self-Collected Vaginal Swab			Aptima Mycoplasma genitalium Assay				Symptom Status	
	Alt TMA Assay #1	Alt TMA Assay #2	Alt TMA Assay #3[1]	Self-Collected Vaginal Swab	Clinician-Collected Vaginal Swab	Endocervical Swab	Urine	Sym	Asym
Not infected	+	-	-	+	+	+	-	2	0
Not infected	-	+	-	+	+	+	+	3	0
Not infected	-	+	-	+	+	+	-	1	2
Not infected	-	+	-	+	+	-	-	0	1
Not infected	-	+	-	+	-	-	-	1	0
Not infected	-	+	-	-	+	-	-	1	1
Not infected	-	+	-	-	-	-	+	1	0
Not infected	-	-	-	-	-	-	-	2	0
Not infected	-	-	N/A	+	+	+	+	4	0
Not infected	-	-	N/A	+	+	+	-	3	1
Not infected	-	-	N/A	+	+	-	-	1	2
Not infected	-	-	N/A	+	-	+	-	1	0
Not infected	-	-	N/A	+	-	-	-	1	0
Not infected	-	-	N/A	-	+	-	-	6	1
Not infected	-	-	N/A	-	-	+	-	2	5
Not infected	-	-	N/A	-	-	-	+	4	1
Not infected	-	-	N/A	-	-	-	-	845	568
Not infected	-	-	N/A	-	-	-	NR	2	2
Not infected	-	-	N/A	-	-	NR	-	5	9
Not infected	-	-	N/A	-	NR	-	+	1	0
Not infected	-	-	N/A	-	NR	-	-	9	11
Not infected	-	-	N/A	-	NR	NR	-	0	3
Not infected	-	-	N/A	NR	-	+	-	0	1
Not infected	-	-	N/A	NR	-	-	-	5	4
Not infected	-	-	N/A	NR	NR	NR	-	0	1

Table 8: Mycoplasma genitalium Patient Infected Status for Female Specimens (continued)

Patient Infected Status	Self-Collected Vaginal Swab			Aptima Mycoplasma genitalium Assay				Symptom Status	
	Alt TMA Assay #1	Alt TMA Assay #2	Alt TMA Assay #3[1]	Self-Collected Vaginal Swab	Clinician-Collected Vaginal Swab	Endocervical Swab	Urine	Sym	Asym
Not infected	-	NR	-	-	-	-	-	6	5
Not infected	NR	-	-	-	-	-	+	1	0
Not infected	NR	-	-	-	-	-	-	22	10
Not infected	NR	-	-	-	-	NR	-	0	1
Not infected	NR	-	-	-	NR	-	-	1	0
Not infected	NR	-	-	NR	-	-	-	0	1

Asym = asymptomatic, N/A = not applicable, NR = no result, Sym = symptomatic.
[1] Alt TMA #3 results are not applicable if results of Alt TMA assays #1 and #2 are in agreement; some samples may have been tested unnecessarily with Alt TMA assay #3.

Table 9: Mycoplasma genitalium Patient Infected Status for Male Specimens

Patient Infected Status	Urethral Swab			Aptima Mycoplasma genitalium Assay			Symptom Status	
	Alt TMA Assay #1	Alt TMA Assay #2	Alt TMA Assay #3[1]	Urethral Swab	Penile Meatal Swab	Urine	Sym	Asym
Infected	+	+	+	+	+	+	1	0
Infected	+	+	N/A	+	+	+	83	49
Infected	+	+	N/A	+	+	-	4	0
Infected	+	+	N/A	+	+	NR	0	1
Infected	+	+	N/A	+	-	+	7	3
Infected	+	+	N/A	+	-	-	3	1
Infected	+	+	N/A	+	NR	-	1	0
Infected	+	+	N/A	-	-	-	1	0
Infected	+	NR	+	+	+	+	1	1
Infected	-	+	+	+	+	-	1	0
Infected	-	+	+	+	-	-	0	1
Infected	-	+	+	-	+	-	1	0
Infected	-	+	+	-	-	-	0	1
Infected	NR	+	+	+	+	+	1	2
Infected	NR	+	+	+	-	+	0	1
Infected	NR	+	+	+	-	-	0	1
Not infected	-	+	-	+	+	-	0	1

Table 9: Mycoplasma genitalium Patient Infected Status for Male Specimens (continued)

Patient Infected Status	Urethral Swab			Aptima Mycoplasma genitalium Assay			Symptom Status	
	Alt TMA Assay #1	Alt TMA Assay #2	Alt TMA Assay #3[1]	Urethral Swab	Penile Meatal Swab	Urine	Sym	Asym
Not infected	-	+	-	+	-	-	0	2
Not infected	-	+	-	-	+	-	1	0
Not infected	-	+	-	-	-	-	2	3
Not infected	-	-	-	-	-	-	1	0
Not infected	-	-	N/A	+	+	-	1	0
Not infected	-	-	N/A	+	-	-	0	2
Not infected	-	-	N/A	-	+	+	1	0
Not infected	-	-	N/A	-	+	-	14	11
Not infected	-	-	N/A	-	-	+	6	2
Not infected	-	-	N/A	-	-	-	721	589
Not infected	-	-	N/A	-	-	NR	0	3
Not infected	-	-	N/A	-	NR	-	0	8
Not infected	-	NR	-	-	+	-	0	1
Not infected	-	NR	-	-	-	-	7	5
Not infected	NR	-	-	-	-	-	8	9

Asym = asymptomatic, N/A = not applicable, NR = no result, Sym = symptomatic.
[1] Alt TMA #3 results are not applicable if results of Alt TMA assays #1 and #2 are in agreement; some samples may have been tested unnecessarily with Alt TMA assay #3.

Specimen-Specific Agreement Analyses

Agreement analysis was performed by comparing Aptima Mycoplasma genitalium Assay results to a composite reference comprised of testing the same specimen type with up to 3 alternate TMA assays, and using the result that is concordant in at least 2 of the 3 TMA assays.

The positive (PPA) and negative (NPA) percent agreement of the Aptima Mycoplasma genitalium Assay for *M. genitalium* detection are shown for all female and male specimens overall in Table 10 and by symptom status in Table 11.

Table 10: Specimen-Specific Agreement

Specimen Type	N	Reference+/ Aptima+	Reference-/ Aptima+	Reference-/ Aptima-	Reference+/ Aptima-	PPA (95% CI)[1]	NPA (95% CI)[1]
CVS	1729	175	17	1534	3	98.3 (95.2-99.4)	98.9 (98.3-99.3)
PVS	1724	173	24	1525	2	98.9 (95.9-99.7)	98.5 (97.7-99.0)
ES	1734	163	7	1559	5	97.0 (93.2-98.7)	99.6 (99.1-99.8)
FU	1774	147	9	1609	9	94.2 (89.4-96.9)	99.4 (98.9-99.7)
US	1563	162	6	1392	3	98.2 (94.8-99.4)	99.6 (99.1-99.8)

Table 10: Specimen-Specific Agreement (continued)

Specimen Type	N	Reference+/ Aptima+	Reference-/ Aptima+	Reference-/ Aptima-	Reference+/ Aptima-	PPA (95% CI)[1]	NPA (95% CI)[1]
PM	1563	162	14	1379	8	95.3 (91.0-97.6)	99.0 (98.3-99.4)
MU	1578	159	2	1413	4	97.5 (93.9-99.0)	99.9 (99.5-100)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FU = female urine, MU = male urine, NPA = negative percent agreement, PM = penile meatal swab, PPA = positive percent agreement, PVS = patient-collected vaginal swab, US = male urethral swab.

[1]Score 95% CI.

Table 11: Specimen-Specific Agreement by Symptom Status

Specimen Type	Symptom Status	N	Reference+/ Aptima+	Reference-/ Aptima+	Reference-/ Aptima-	Reference+/ Aptima-	PPA (95% CI)[1]	NPA (95% CI)[1]
CVS	Sym	1050	123	12	913	2	98.4 (94.4-99.6)	98.7 (97.7-99.3)
	Asym	679	52	5	621	1	98.1 (90.1-99.7)	99.2 (98.1-99.7)
PVS	Sym	1047	121	18	908	0	100 (96.9-100)	98.1 (96.9-98.8)
	Asym	677	52	6	617	2	96.3 (87.5-99.0)	99.0 (97.9-99.6)
ES	Sym	1057	115	4	935	3	97.5 (92.8-99.1)	99.6 (98.9-99.8)
	Asym	677	48	3	624	2	96.0 (86.5-98.9)	99.5 (98.6-99.8)
FU	Sym	1074	106	7	955	6	94.6 (88.8-97.5)	99.3 (98.5-99.6)
	Asym	700	41	2	654	3	93.2 (81.8-97.7)	99.7 (98.9-99.9)
US	Sym	866	102	1	761	2	98.1 (93.3-99.5)	99.9 (99.3-100)
	Asym	697	60	5	631	1	98.4 (91.3-99.7)	99.2 (98.2-99.7)
PM	Sym	870	101	8	756	5	95.3 (89.4-98.0)	99.0 (97.9-99.5)
	Asym	693	61	6	623	3	95.3 (87.1-98.4)	99.0 (97.9-99.6)
MU	Sym	874	99	2	770	3	97.1 (91.7-99.0)	99.7 (99.1-99.9)
	Asym	704	60	0	643	1	98.4 (91.3-99.7)	100 (99.4-100)

Asym = asymptomatic, CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FU = female urine, MU = male urine, NPA = negative percent agreement, PM = penile meatal swab, PPA = positive percent agreement, PVS = patient-collected vaginal swab, Sym = symptomatic, US = male urethral swab.

[1] Score 95% CI.

Reproducibility

Aptima Mycoplasma genitalium Assay reproducibility was evaluated on the Panther System at 3 US sites using 6 panel members. Two (2) operators performed testing at each site. Each operator performed 1 run per day over 5 days using 1 reagent lot over the course of testing. Each run had 3 replicates of each panel member.

The 2 negative panel members consisted of *M. genitalium*-negative urine transport medium (UTM) or simulated vaginal matrix (SVM). The positive panel members were created by spiking the UTM and SVM matrices with 1-2X LoD (low-positive) or 2-3X LoD (moderate positive) concentrations of *M. genitalium*-positive whole cell lysates.

The agreement with expected results was 100% for all panel members.

Table 12 shows the signal variability of assay S/CO results for each panel member between sites, between operators, between days, between runs, within runs, and overall. Only samples with valid results were included in the analyses.

Table 12: Reproducibility Study Data: Signal Variability by Panel Member

Panel Description	N	Mean S/CO	Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
UTM Negative	90	0.00	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
UTM Low Pos	90	24.64	0.45	1.82	0.00	0.00	0.43	1.74	0.43	1.74	2.38	9.67	2.59	10.51
UTM Mod Pos	90	25.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.20	4.71	1.41	5.51
SVM Negative	90	0.00[1]	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
SVM Low Pos	90	24.05	0.00	0.00	0.48	1.98	0.00	0.00	0.00	0.00	1.85	7.67	2.12	8.83
SVM Mod Pos	90	25.14	0.00	0.00	0.48	1.91	0.56	2.25	0.56	2.25	1.14	4.53	1.65	6.58

CV = coefficient of variation, Mod = moderate, NC = not calculable, Pos = positive, S/CO = signal to cutoff, SD = standard deviation, SVM = simulated vaginal matrix, UTM = urine transport medium.

Note: In case variability from some factors may be numerically negative, SD and CV are shown as 0.00.

[1] 1.1% (1 out of 90) results had S/CO value of 0.03 and 98.9% (89 out of 90) results had S/CO value of 0.

Panther System Analytical Performance

Within Laboratory Precision Study

The precision of the Aptima Mycoplasma genitalium Assay on the Panther System was assessed at Hologic. The study was conducted with 2 Panther instruments, 2 operators, and 3 lots of reagents over 12 days. The panels used in the study consisted of negative, low positive, and moderate positive urine and simulated vaginal swab samples. Positive panels were created by spiking *M. genitalium* whole cell lysate into negative sample matrices. The concentrations of the positive panel members are shown in Table 13 along with the study results. Variability between Panther instruments, operators, reagent lots, and between and within runs is shown as SD and %CV.

Table 13: Precision of the Aptima Mycoplasma genitalium Assay

				Between Instruments		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
Panel	N	% Detected[1]	Mean S/CO	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Urine:UTM	240	100[2]	0.0	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
1.5X LoD Urine:UTM	240	100	24.7	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.81	0.47	1.88	2.08	8.43	2.14	8.68
3X LoD Urine:UTM	240	100	25.2	0.08	0.33	0.16	0.62	0.00	0.00	0.27	1.05	0.19	0.76	1.36	5.38	1.41	5.58
Negative SVM	240	100[2]	0.0	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
1.5X LoD SVM	240	98.3	23.7	0.00	0.00	0.00	0.00	0.36	1.51	0.13	0.54	0.00	0.00	3.83	16.13	3.84	16.21
3X LoD SVM	240	100	24.8	0.14	0.58	0.45	1.84	0.00	0.00	0.46	1.87	0.63	2.56	1.18	4.75	1.49	6.02

CV = coefficient of variation, LoD, limit of detection, NC= not calculable, S/CO = signal to cutoff, SD = standard deviation, SVM = simulated vaginal matrix, UTM = urine transport medium.

[1] Detected defined as S/CO > 1.0.

[2] 100% *M. genitalium* negative.

Analytical Sensitivity

Sensitivity panels were prepared with 2 strains of *M. genitalium* (1 azithromycin resistant and 1 azithromycin susceptible) using pooled negative male and female urine, vaginal swabs, and penile meatal swabs. LoD study testing included the use of 2 reagent lots and was performed on 2 Panther Systems. LoD (in genome equivalents (GE)/mL), defined as the target concentration that can be detected in 95% of the replicates tested for each specimen is shown in Table 14.

Table 14: Limit of Detection of the Aptima Mycoplasma genitalium Assay

	***Mycoplasma genitalium* LoD (GE/mL)**	
Specimen Type	Strain 1	Strain 2
Vaginal swab	0.04	0.10
Female urine	0.04	0.12
Penile meatal swab	0.05	0.10

Table 14: Limit of Detection of the Aptima Mycoplasma genitalium Assay (continued)

	***Mycoplasma genitalium* LoD (GE/mL)**	
Specimen Type	**Strain 1**	**Strain 2**
Male urine	0.03	0.16

Inclusivity

Nine (9) strains of *M. genitalium*, representing both macrolide antibiotic resistant and susceptible strains, were spiked into pools of specimen matrix derived from male and female urine, vaginal swab, and penile meatal swab specimens. Testing was performed in triplicate using 3 Panther Systems using 3 lots of reagents. Seven (7) of the 9 strains were detected at ≥95% positivity at ≤0.29-0.49 GE/mL in all 4 specimen types. One (1) strain was ≥95% positive at 0.85-1.46 GE/mL in each of the 4 specimen types. The remaining strain was detected at 100% positive at 1.16 and 1.46 GE/mL in vaginal and penile meatal swabs, respectively, 100% positive at 3.47 GE/mL in female urine, and 100% positive at 8.50 GE/mL in male urine.

Cross-Reactivity in the Presence of Microorganisms

Cross-reactivity 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 swab and urine samples for each isolate. The list of organisms and the concentrations tested are provided in Table 15. No cross-reactivity in the Aptima Mycoplasma genitalium Assay was observed with any of the organisms tested.

An *in silico* analysis was performed to determine if the oligonucleotides (amplification primers and detection probes) in the Aptima Mycoplasma genitalium Assay could amplify and detect nucleic acid sequences from the following organisms: Human papillomavirus (HPV) type 31, HPV type 35, HPV type 54, *Mycobacterium smegmatis*, *Chlamydia trachomatis* serovars L1, L2, L3, and *Treponema pallidum*. Using BLAST methodology, no significant interactions were detected.

The Aptima Mycoplasma genitalium Assay was also evaluated by testing the same organisms (Table 15) in swab and urine samples spiked with *M. genitalium* lysate to a final concentration of 3X LoD for each sample type (at least 3 replicates of each isolate). The Aptima Mycoplasma genitalium Assay result was not significantly affected by the presence of microorganisms tested, except in the presence of *Mycoplasma pneumoniae* (where lower signal outputs were observed). *M. pneumoniae* is most commonly found in the lower respiratory tract.

Table 15: Microorganisms Tested in the Aptima Mycoplasma genitalium Assay on the Panther System

Microorganism	Concentration	Microorganism	Concentration
Acinetobacter lwoffii	$1x10^6$ CFU/mL	HPV type 18 (HeLa cells)	$1x10^4$ cells/mL
Actinomyces israelii	$1x10^6$ CFU/mL	HPV type 58	$1x10^4$ copies/mL
Alcaligenes faecalis	$1x10^6$ CFU/mL	HPV type 39	$1x10^4$ copies/mL
Atopobium vaginae	$1x10^9$ rRNA copies/mL	HPV type 51	$1x10^4$ copies/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Klebsiella pneumoniae*	$1x10^6$ CFU/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^6$ CFU/mL

Table 15: Microorganisms Tested in the Aptima Mycoplasma genitalium Assay on the Panther System (continued)

Microorganism	Concentration	Microorganism	Concentration
Campylobacter jejuni	$1x10^6$ CFU/mL	*Lactobacillus crispatus*	$1x10^6$ CFU/mL
Candida albicans	$1x10^6$ CFU/mL	*Leptotrichia buccalis*	$1x10^5$ CFU/mL
Chlamydia trachomatis	$1x10^4$ IFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Megasphaera* type 1	$1x10^9$ copies/mL
Chromobacterium violaceum	$1x10^6$ CFU/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Mobiluncus mulieris*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^9$ copies/mL
Cytomegalovirus	$2.5 x10^4$ TCID 50/mL	*Mycoplasma pneumoniae*	$1x10^6$ CFU/mL
Elizabethkingia meningosepticum	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Pentatrichomonas hominis*	$1x10^5$ cells/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Prevotella bivia*	$1x10^6$ CFU/mL
Escherichia coli	$1x10^6$ CFU/mL	*Propionibacterium acnes*	$1x10^6$ cells/mL
Finegoldia magna	$1x10^9$ copies/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Fusobacterium nucleatum	$1x10^6$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Gardnerella vaginalis	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Herpes simplex virus type 1	$2.5 x10^3$ TCID 50/mL	*Staphylococcus saprophyticus*	$1x10^6$ CFU/mL
Herpes simplex virus type 2	$2.5 x10^3$ TCID 50/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
HIV-1	$1x10^6$ copies/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
HPV type 6	$1x10^6$ copies/mL	*Trichomonas vaginalis*	$1x10^5$ cells/mL
HPV type 11	$1x10^8$ copies/mL	*Ureaplasma parvum*	$1x10^9$ rRNA copies/mL
HPV type 16 (SiHa cells)	$1x10^4$ cells/mL	*Ureaplasma urealyticum*	$1x10^9$ rRNA copies/mL

Interference

Personal lubricants, deodorants, spermicides, antifungals, antibiotics, antivirals, and seminal fluid were spiked into swab and urine samples at final concentrations of 1% (vol/vol or wt/vol), porcine gastric mucus at 0.03% (wt/vol), leukocytes at $4x10^5$ cells/mL, and whole blood at 5% (vol/vol). Urine was tested at high and low pH, and to test the effect of urine metabolites KOVA-Trol® High Abnormal with Urobilinogen urinalysis control was diluted into UTM in place of urine.

Substances were diluted in the matrix where they would be found (i.e., women's health products in vaginal swabs, ingested medication in urine).

Interference was not observed with any of the substances at the concentration listed above when spiked with *M. genitalium* whole cell lysate to a final concentration of 3X LoD for each sample type and tested in the Aptima Mycoplasma genitalium Assay.

Interference in assay results was observed when mucus at a final concentration of 0.3% w/v was added to clinical specimen matrix. Interference was not observed when mucus at a final concentration of 0.03% w/v was added to clinical specimen matrix.

Carryover

To assess the amount of carryover contamination with the assay on the Panther System, an analytical study was conducted where *M. genitalium* negative and *M. genitalium* high positive samples were tested in a checkerboard pattern of alternating negative and positive samples. The positive samples consisted of $6.1x10^6$ GE/mL *M. genitalium* in simulated vaginal swab samples; the negative samples were simulated vaginal swab samples without *M. genitalium*. The checkerboard arrangement was tested on 3 Panther instruments, 4 runs/instrument, 40 negative and 40 positive samples/run with 1 reagent lot. No false positive results were observed in any of the runs.

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