Aptima® Chlamydia trachomatis Assay (Panther System)

For *in vitro* diagnostic use.

Rx Only.

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General Information

Intended Use

The Aptima® Chlamydia trachomatis (CT) Assay is an *in vitro* qualitative nucleic acid amplification test (NAAT) for the detection of ribosomal RNA (rRNA) from *Chlamydia trachomatis* to aid in the diagnosis of chlamydial urogenital disease using the Panther® System.

The assay may be used to test the following specimens from symptomatic or asymptomatic individuals: patient-collected vaginal swab specimens (in a clinical setting); and female and male urine specimens.

[1]Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The Aptima® Multitest Swab Specimen Collection Kit has not been evaluated for home use.

Summary and Explanation of the Test

Chlamydia trachomatis infections are one of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 1,644,416 (495.5 cases per 100,000 population) new cases of CT infections were reported to the Centers for Disease Control and Prevention (CDC) in 2021 (1).

Chlamydiae are nonmotile, gram-negative, obligate intracellular bacteria. The CT species is comprised of fifteen serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2 and L3) that can cause disease in humans (2). The serovars D through K are the major cause of genital chlamydial infections in men and women (4). *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and pelvic inflammatory disease (4, 5, 6). *C. trachomatis* infections are often asymptomatic in both males and females (7). Children born to infected mothers are at significantly higher risk for inclusion conjunctivitis and chlamydial pneumonia (8, 9, 10).

Cell culture was once considered to be the “gold standard” for detection of CT. Culture is quite specific, but published data demonstrate that nucleic acid amplification tests (NAATs) have a higher clinical sensitivity than culture with similar specificity (11, 12, 13). Methodologies employing NAATs are now considered the gold standard for CT detection (14).

The Aptima CT Assay is a NAAT that utilizes target capture, Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (HPA) technologies to streamline specimen processing, amplify target rRNA, and detect amplicon, respectively. Studies comparing performance and specimen inhibition of various amplification systems have demonstrated the benefits of the target capture, TMA, and HPA technologies used in the Aptima CT Assay (15, 16). The Aptima CT Assay targets different nucleic acid sequences than those targeted by other *C. trachomatis* NAATs, including the Aptima Combo 2® Assay.

Principles of the Procedure

The Aptima CT Assay involves target capture, transcription-mediated amplification (Hologic® TMA), and hybridization protection assay (HPA) technologies.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the Aptima CT Assay is performed in the laboratory, the target rRNA

molecule is isolated from the specimens using a capture oligomer via target capture that utilizes magnetic microparticles. 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 reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Hologic TMA reaction replicates a specific region of the 16S rRNA from CT via DNA intermediates. A unique set of primers is used for the target molecule. Detection of the rRNA amplification product sequences (amplicon) is achieved using nucleic acid-based hybridization protection assay. A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU).

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

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

D. To reduce the risk of invalid results, carefully read the entire package insert and refer to the *Panther/Panther Fusion System Operator's Manual* for procedural information prior to performing the assay on the Panther System.

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

Laboratory Related

F. Use only supplied or specified disposable laboratory ware.

G. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

H. **Warning: Irritant and Corrosive:** Avoid contact of Auto Detect 2 with skin, eyes and mucous membranes. If this fluid comes into contact with skin or eyes, wash with water. If this fluid spills, dilute the spill with water before wiping dry.

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

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

K. Use good standard practices for molecular laboratories including environmental monitoring. See *Procedural Notes* for suggested Lab Contamination Monitoring Protocol for the Panther System.

Specimen Related

L. This assay has been tested using vaginal swab specimens, and female and male urine specimens only. Performance with specimens other than those specified under *Specimen Collection and Storage* has not been evaluated.

M. Expiration dates listed on the collection kits pertain to the collection site and not the testing facility. Samples collected any time prior to the expiration date of the collection kit and transported and stored in accordance with the package insert, are valid for testing even if the expiration date on the collection tube has passed.

N. 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.

O. 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.

P. Avoid cross-contamination by discarding used materials without passing them over any other container.

Q. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.

R. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another during specimen handling in the laboratory. Change gloves if they come in contact with a specimen.

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

T. Upon piercing, liquid can discharge from Aptima transport tube caps under certain conditions. Follow instructions in the *Panther System Test Procedure* to prevent this occurrence.

Assay Related

U. 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.

V. Use Universal Precautions when handling controls.

W. Avoid microbial and ribonuclease contamination of reagents.

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

Y. Do not interchange, mix, or combine assay reagents from kits with different lot numbers. Aptima controls and assay fluids (Panther System) can be from different lot numbers.

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

AA. Some reagents of this kit are labeled with hazard information.

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

US Hazard Information

Selection Reagent
Boric Acid 1 - 5%

WARNING
H315 - Causes skin irritation
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection

Reagent Storage and Handling Requirements

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

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2ºC to 8ºC	N/A	N/A
Enzyme Reagent	2ºC to 8ºC	N/A	N/A
Probe Reagent	2ºC to 8ºC	N/A	N/A
Target Capture Reagent B	2ºC to 8ºC	N/A	N/A
Amplification Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Enzyme Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Probe Reconstitution Solution CT	2ºC to 30ºC	2ºC to 8ºC	60 days
Selection Reagent	2ºC to 30ºC	2ºC to 30ºC	60 days
Target Capture Reagent	15ºC to 30ºC	15ºC to 30ºC	60 days
Positive Control, CT / Negative Control GC	2ºC to 8ºC	N/A	Single Use Vial
Positive Control, GC/ Negative Control CT	2ºC to 8ºC	N/A	Single Use Vial

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

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

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

E. Discard any unused reconstituted reagents and wTCR after 60 days or after the Master Lot expiration date, whichever comes first.

F. Controls are stable until the date indicated on the vials.

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

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

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

J. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

K. Do not freeze the reagents.

Specimen Collection and Storage

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

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

The Aptima CT Assay is designed to detect the presence of *C. trachomatis* in patient-collected vaginal swab specimens, and female and male urine specimens. Performance with specimens other than those collected with the following specimen collection kits has not been evaluated:

- Aptima Multitest Swab Specimen Collection Kit
- Aptima Urine Collection Kit for Male and Female Urine Specimens

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, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested. Specimens must be assayed with the Aptima CT Assay within 60 days of collection. If longer storage is needed, freeze specimens in the swab specimen transport tube within 7 days of collection at –20°C to –70°C to allow testing up to 12 months after collection.
2. Urine Specimens
   a. Maintain urine specimen at 2°C to 30°C after collection and transfer to the Aptima Urine Specimen Transport Tube within 24 hours of collection. Transport to the lab in the primary collection container or the transport tube at 2°C to 30°C. Store at 2°C to 30°C and test the processed urine specimens with the Aptima CT Assay within 30 days of collection.
   b. If longer storage is needed, freeze urine specimens in the Aptima Urine Specimen Transport Tube within 7 days of collection at –20°C to –70°C to allow testing up to 12 months after collection.

C. Specimen Storage After Testing

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen transport tubes should be covered with a new, clean plastic film, foil barrier, or cap.

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

3. If assayed samples need to be frozen or shipped, remove the penetrable caps and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained.
4. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all

of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination.**

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

Panther System

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

Reagents and Materials Provided

Aptima Chlamydia trachomatis Assay Kit, 100 tests (2 boxes and 1 Controls kit) (Cat. No. 302925)

Aptima Chlamydia trachomatis Assay Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
TCR-B	**Target Capture Reagent B** *Non-infectious nucleic acids in a buffered solution containing < 5% detergent.*	1 x 0.30 mL

Aptima Chlamydia trachomatis Assay Room Temperature Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	**Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 11.9 mL
ER	**Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 6.3 mL
PR	**Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 15.2 mL
S	**Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 43.0 mL
TCR	**Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 26.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

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

Symbol	Component	Quantity
PCT/ NGC	**Positive Control, CT / Negative Control, GC** *Non-infectious CT nucleic acid in a buffered solution containing < 5% detergent.*	5 x 1.7 mL
PGC/ NCT	**Positive Control, GC / Negative Control, CT** *Non-infectious GC nucleic acid in a buffered solution containing < 5% detergent.*	5 x 1.7 mL

Materials Required But Available Separately

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

	Cat. No.
Panther System	303095
Panther Fusion System	PRD-04172
Panther System Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable *Not all products are available in all regions. Contact your representative for region-specific information*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Urine Specimen Collection Kit for Male and Female Urine Specimens	301040
Aptima Urine Specimen Transport Tubes	105575
Bleach, 5% to 8.25% (0.7M to 1.16M) sodium hypochlorite solution	—
Disposable gloves	—
Aptima penetrable caps	105668
Replacement non-penetrable caps	103036A

		Cat. No.
Replacement caps for the 100-test kits		—
Amplification, Enzyme, and Probe reagent reconstitution solutions	*CL0041 (100 caps)*	
TCR and Selection reagent	*501604 (100 caps)*	

Optional Materials

	Cat. No.
Aptima Controls Kit	301110
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Tube Rocker	—
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens* **used for lab contamination monitoring*	301041

Panther System Test Procedure

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

A. Work Area Preparation

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

B. Reagent Reconstitution/Preparation of a New Kit

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

1. Prior to testing, Amplification, Enzyme, and Probe Reagents must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.
   b. Pair each reconstitution solution with its lyophilized reagent. Before attaching the reconstitution collar, ensure that the reconstitution solution and reagent have matching label symbols.
   c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired. Label caps of reconstitution solution bottles.

d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 1, Step 1).

e. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.

f. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 1, Step 2).

g. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

h. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (Figure 1, Step 4).

i. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the reconstitution solution bottle.

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

k. Recap the plastic bottle with either the saved labeled cap that corresponds to the reagent or new cap. Do not mismatch caps. Record the operator initials and reconstitution date on the label (Figure 1, Step 7).

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

m. Thoroughly mix each reagent by gently inverting prior to loading onto the Panther System. Avoid creating foam when inverting reagents. This step is not required if reagents are loaded onto the system direction after mixing on the tube rocker.

Option: Additional mixing of the Amplification, Enzyme and Probe Reagents using a tube rocker 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.*

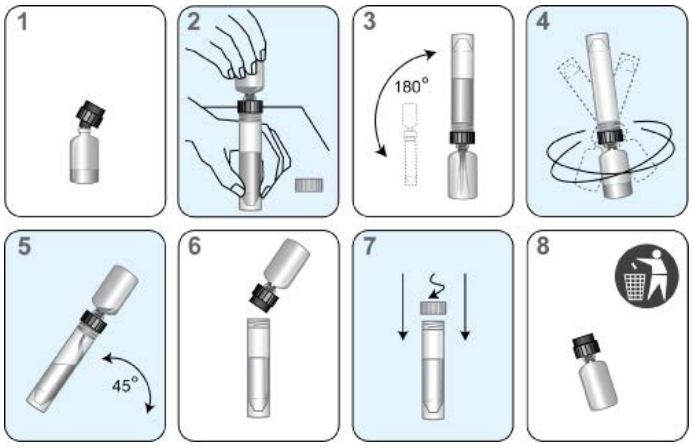

Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

a. Pair the appropriate bottles of TCR and TCR-B.
b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
d. Open the bottle of TCR-B and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the TCR-B bottle.
e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
f. Record operator initials and the current date on the label.
g. Discard the TCR-B bottle and cap.

3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure that it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents

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

   Option: The reconstituted Amplification, Enzyme, and Probe Reagents capped plastic bottles may be placed on a tube rocker set at a moderate speed and tilt for a minimum of 25 minutes to ensure reagents reach room temperature and are thoroughly mixed.

2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.

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

D. Control Preparation

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

E. Specimen Handling

1. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single pink Aptima collection swab in a multitest swab specimen transport tube.

b. A final volume of urine between the black fill lines of a urine specimen transport tube.

2. Allow the specimens to reach room temperature (15°C to 30°C) prior to processing.

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

3. Inspect specimen tubes before loading into the rack:
   a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.
   c. If the liquid level in a urine specimen tube is not between the two black indicator lines on the label, the specimen must be rejected. Do not pierce an overfilled tube.
   d. If a urine specimen tube contains precipitate, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, visually ensure that the precipitate does not prevent delivery of the specimen.

Note: *Failure to follow Steps 3a–c may result in liquid discharge from the specimen tube cap.*

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

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes* for the Panther System.

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

2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software on the Panther System, one pair of controls is required. The Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control CT tubes can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Patient specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of controls is currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated assay reagent kit up to 24 hours **unless:**
   a. Controls are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.

3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

B. Temperature

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

C. Glove Powder

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

D. Lab Contamination Monitoring Protocol for the Panther System

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

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

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing if using the Aptima Unisex Swab Specimen Collection Kit) from its packaging, wet the swab in the Specimen Transport Medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.
7. Test samples with the Aptima CT 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.

Test Interpretation — QC/Patient Results

A. Test Interpretation

Assay test results are automatically interpreted by the Panther System Aptima CT Assay software. A test result may be negative, equivocal, positive, or invalid as determined by total RLU in the detection step (see below). A test result may be invalid due to RLU values outside the normal expected ranges. Initial equivocal and invalid test results should be retested. Report the first valid result. If the result is invalid upon retest, a new specimen should be collected.

Test Interpretation	Total RLU (x1000)
Negative	0* to < 50
Equivocal	50 to < 100
Low RLU Positive[1]	100 to < 5,000
Positive	5,000 to < 12,000
Invalid	0* or > 12,000

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

[1] In the low positive range, data suggest positive results should be interpreted carefully, with the understanding that the likelihood of a false positive may be higher than a true positive.

B. Quality Control Results and Acceptability

The Negative Control for CT, which is labeled "CONTROL + GC PGC / CONTROL – CT NCT," and the Positive Control for CT, which is labeled "CONTROL + CT PCT / CONTROL – GC NGC," act as controls for the target capture, amplification, and detection steps of the assay. In accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations, additional controls for cell lysis and RNA stabilization may be included. The Negative Control for CT, which is labeled "CONTROL + GC PGC / CONTROL – CT NCT," contains non-infectious *N.* gonorrhoeae rRNA. If desired, additional controls can be ordered as a kit. See *Optional Materials*. Correct preparation of specimens is confirmed visually by the presence of a single Aptima collection swab in a swab specimen transport tube or a final volume of urine in between the black fill lines of a urine specimen transport tube.

The Controls must produce the following test results:

Control	Total RLU (x1000)	CT Result
Positive Control, GC/ Negative Control, CT	0* and < 50	Negative
Positive Control, CT/ Negative Control, GC	≥ 100 and < 12,000	Positive

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

1. Each laboratory should implement appropriate control procedures to satisfy the requirements of CLIA regulations.
2. Negative controls may not be effective in monitoring random carryover. See *Panther System Analytical Performance* for results from a high-target analytical carryover study that was performed to demonstrate control of carryover on the Panther System.

C. Specimen Preparation Control (optional)

The Negative Control for CT, which is labeled "CONTROL + GC PGC / CONTROL – CT NCT," and the Positive Control for CT, which is labeled "CONTROL + CT PCT / CONTROL – GC NGC," act as controls for the target capture, amplification, and detection steps of the assay and must be included in each assay run. If desired, controls for cell lysis and RNA stabilization can be tested in accordance with the requirements of appropriate accrediting organizations or individual laboratory procedures. Known positive specimens can serve as controls by being prepared and tested in conjunction with unknown specimens. Specimens used as preparation controls must be stored, handled, and tested according to the package insert. Specimen preparation controls should be interpreted in the same manner as described for patient test specimens. See *Test Interpretation — QC/Patient Results*, *Patient Test Results*.

D. Patient Test Results

1. If the controls in any run do not yield the expected results, test results on patient specimens in the same run must not be reported.
2. Swab and urine specimen results. See *Notes* below.
   a. Initial results

CT Pos*	Positive for CT rRNA.
CT Neg	Presumed negative for CT rRNA.
CT Equiv	Sample should be retested.
Invalid	Sample should be retested.

   b. Retest results

CT Pos*	Positive for CT rRNA.
CT Neg	Presumed negative for CT rRNA.
CT Equiv	Indeterminate, a new specimen should be collected.
Invalid	Indeterminate, a new specimen should be collected.

*Low RLU Positive specimen results are included in this category. See *Test Interpretation — QC/Patient Results* above.

Notes

- The first valid, non-equivocal result for each analyte is the result that should be reported.
- Careful consideration of performance data is recommended for interpreting Aptima CT test results for asymptomatic individuals or any individuals in low prevalence populations.
- A negative result does not preclude the presence of a CT infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient rRNA to be detected. Test results may be affected by improper specimen collection, improper specimen storage, technical error, specimen mix-up, or target levels below the assay limit of detection.

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 package insert may result in erroneous results.

B. The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of CT.

C. Urine and vaginal 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. The Aptima CT Assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications.

E. Reliable results are dependent on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, proper specimen collection techniques are necessary. Refer to package insert of the appropriate Aptima Specimen Collection Kit.

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

G. Results from the Aptima CT Assay should be interpreted in conjunction with other laboratory and 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 CT 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. For the vaginal swab and urine specimen clinical study, performance for detecting CT is derived from high prevalence populations. Positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.

K. Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated.

L. The patient-collected specimen application is limited to clinical settings where support/ counseling is available to explain the procedures and precautions.

M. The Aptima CT Assay has not been validated for use with vaginal swab specimens collected by patients at home.

N. The performance of the Aptima CT Assay has not been evaluated in adolescents less than 14 years of age.

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

P. Customers must independently validate an LIS transfer process.

Q. First catch urine specimens are acceptable but may detect up to 10% fewer CT infections when compared with vaginal swab specimens. (17)

Panther System Expected Values

Estimates of CT in patient populations depend on the sensitivity of the test used to detect infections and on patient risk factors such as age, lifestyle, and the presence or absence of symptoms. A summary of the positivity of CT, as determined by the Aptima CT Assay on the Panther System, is shown in Table 1 for the multicenter study, by clinical site and overall.

Table 1: Positivity of CT as Determined by the Aptima CT Assay in Patient-collected Vaginal Swab, Female Urine, and Male Urine Specimens by Clinical Site

Site	Positivity % (# positive/# tested with valid non-equivocal results)		
	PVS	FU	MU
1	36.4 (8/22)	45.5 (10/22)	11.9 (21/177)
2	3.1 (12/385)	2.6 (10/385)	0.8 (3/373)
3	6.5 (5/77)	3.9 (3/77)	3.3 (2/61)
4	20.0 (1/5)	0 (0/5)	0 (0/13)
5	11.2 (29/258)	8.3 (21/253)	7.6 (31/409)
6	10.7 (53/494)	9.5 (46/484)	16.3 (50/307)
7	16.8 (42/250)	16.7 (41/246)	10.2 (23/226)
8	5.5 (6/110)	3.6 (4/111)	6.3 (2/32)
9	2.5 (8/314)	2.3 (6/260)	0.9 (2/221)
10	7.5 (19/253)	6.8 (17/251)	13.2 (12/91)
11	3.1 (3/97)	2.2 (2/92)	0 (0/54)
All	8.2 (186/2265)	7.3 (160/2186)	7.4 (146/1964)

FU = female urine; **MU** = male urine; **PVS** = patient-collected vaginal swab.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive (PPV) and negative predictive values (NPV) of the Aptima CT Assay across different hypothetical prevalence rates are shown for patient-collected vaginal swab and male urine specimens in Table 2. For each specimen type, the PPV and NPV are derived for different hypothetical prevalence rates using the overall sensitivity and specificity estimates from the multi-center clinical study (See Table 3).

Table 2: Positive and Negative Predictive Values for Hypothetical Prevalence Rates by Specimen Type

Specimen Type		Hypothetical Prevalence						
		1%	2%	5%	10%	15%	20%	25%
PVS	PPV (%)	66.7	80.2	91.2	95.6	97.2	98.0	98.5
	NPV (%)	100	99.9	99.7	99.5	99.1	98.8	98.4
MU	PPV (%)	78.4	88.0	95.0	97.6	98.4	98.9	99.2
	NPV (%)	100	100	99.9	99.8	99.8	99.7	99.5

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

Panther System Clinical Performance

Clinical Study

A prospective, multicenter clinical study was conducted to establish the clinical performance characteristics of the Aptima CT Assay on the Panther System. Specimens were collected from 4413 symptomatic and asymptomatic women and men enrolled at 11 geographically and ethnically diverse US clinical sites, including obstetrics and gynecology, family planning, and STI clinics. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. One hundred sixty six (166) enrolled subjects were not evaluable (28 were withdrawn and 138 did not have at least one specimen with a valid non-excluded Aptima CT Assay result and a conclusive infected status). Of the 4247 evaluable subjects, 2283 were women and 1964 were men. The average age among evaluable study subjects was 34.5 years (range = 14 to 84 years). Symptoms were reported in 45.7% (1939/4247) of the evaluable subjects.

Up to 5 specimens were collected from each female subject (4 patient-collected vaginal swabs, 1 first-catch urine), and 1 first-catch urine specimen was collected from each male subject. All specimens were collected by the subject at the clinical sites.

Specimens were tested with the Aptima CT Assay on the Panther System. Specimens with initial equivocal or invalid Aptima CT Assay results or instrument processing errors were retested, volume permitting; valid retest results were included in the performance analyses. Patient-collected vaginal swabs and male and female urine specimens were tested with up to 3 FDA-cleared CT NAATs to establish the specimen-specific composite comparator method result as follows:

- Male urine: The patient infected status (PIS) was derived from male urine specimens.
- Female urine: The composite comparator algorithm (CCA) interpretation was derived from female urine specimens.
- Vaginal swab: The PIS was derived from vaginal swab and female urine specimens.

Performance of the Aptima CT Assay was estimated relative to the PIS and reported as sensitivity and specificity for vaginal swab and male urine specimens, whereas performance of the Aptima CT Assay was estimated relative to the CCA and reported as positive and negative percent agreement (PPA and NPA) for female urine specimens.

Of the specimens collected, 6592 were processed in valid Aptima CT Assay runs, including 213 (3.2%) that had to be retested due to invalid results. Overall, 6561 (99.5%) had final valid results, and 31 (0.5%) had final invalid results and were excluded from the analyses. A total of 6415 specimens from 4247 evaluable subjects were included in the analyses comparing Aptima CT Assay results to the specimen-specific PIS or CCA interpretations: 2265 patient-collected vaginal swab, 2186 female urine, and 1964 male urine specimens.

Performance Results

Performance characteristics of the Aptima CT Assay were estimated for each specimen type. Table 3 shows the sensitivity, specificity, PPV, and NPV of the Aptima CT Assay on the Panther System and the prevalence of CT in patient-collected vaginal swab and male urine specimens based on the specimen-specific PIS. Table 4 shows the PPA and NPA of the assay in female urine specimens based on CCA.

Table 3: Performance Characteristics of the Aptima CT Assay by Symptom Status in Patient-collected Vaginal Swab and Male Urine Specimens Compared to Patient Infected Status

Specimen Type	Symptom Status	N	TP	FP[1]	TN	FN[2]	Prev %	Sensitivity (95% CI)[3]	Specificity (95% CI)[3]	PPV % (95% CI)[4]	NPV % (95% CI)[4]
PVS	All	2265	176	10	2070	9	8.2	95.1 (91.0–97.4)	99.5 (99.1–99.7)	94.6 (91.0–97.6)	99.6 (99.3–99.8)
	Sym	1102	89	6[a]	1001	6[a]	8.6	93.7 (86.9–97.1)	99.4 (98.7–99.7)	93.7 (88.4–98.0)	99.4 (98.9–99.8)
	Asym	1163	87	4[b]	1069	3[b]	7.7	96.7 (90.7–98.9)	99.6 (99.0–99.9)	95.6 (91.0–99.0)	99.7 (99.3–100)
MU	All	1964	141	5	1816	2	7.3	98.6 (95.0–99.6)	99.7 (99.4–99.9)	96.6 (93.4–99.3)	99.9 (99.7–100)
	Sym	828	85	4[c]	738	1[c]	10.4	98.8 (93.7–99.8)	99.5 (98.6–99.8)	95.5 (90.8–99.0)	99.9 (99.6–100)
	Asym	1136	56	1[d]	1078	1[d]	5.0	98.2 (90.7–99.7)	99.9 (99.5–100)	98.2 (94.0–100)	99.9 (99.7–100)

Asym = asymptomatic; **CI** = confidence interval; **FN** = false negative; **FP** = false positive; **MU** = male urine; **Prev** = prevalence; **PVS** = patient-collected vaginal swab; **Sym** = symptomatic; **TN** = true negative; **TP** = true positive.

[1]Specimens of the same type, unless noted otherwise, were also tested by an alternative *C. trachomatis* NAAT assay with the following results (# positive results / # samples tested): [a]1/6, [b]1/4, [c]0/4, [d]0/1.

[2]Specimens of the same type, unless noted otherwise, were also tested by an alternative *C. trachomatis* NAAT assay with the following results (# negative results / # samples tested): [a]1/6, [b]1/3, [c]1/1, [d]0/1.

[3]Score CI.

[4]Percentile CI obtained using the bootstrap re-sampling method with 2000 iterations.

Table 4: Performance Characteristics of the Aptima CT Assay in Female Urine Specimens by Symptom Status Compared to the CCA

Specimen Type	Symptom Status	N	CCA+/ ACT+	CCA-/ ACT+[1]	CCA-/ ACT-	CCA+/ ACT-[2]	PPA (95% CI)[3]	NPA (95% CI)[3]
FU	All	2186	151	9	2023	3	98.1 (94.4–99.3)	99.6 (99.2–99.8)
	Sym	1050	74	7[a]	968	1[a]	98.7 (92.8–99.8)	99.3 (98.5–99.7)
	Asym	1136	77	2[b]	1055	2[b]	97.5 (91.2–99.3)	99.8 (99.3–99.9)

ACT = Aptima Chlamydia trachomatis Assay; **CCA** = composite comparator algorithm; **CI** = confidence interval; **FU** = female urine; **NPA** = negative percent agreement; **PPA** = positive percent agreement.

[1]Female urine specimens were also tested by an alternative CT NAAT assay with the following results (# positive results / # samples tested): [a]2/7, [b]0/2.

[21]Female urine specimens were also tested by an alternative CT NAAT assay with the following results (# positive results / # samples tested): [a]1/1, [b]2/2.

[3]Score CI.

Table 5 shows the sensitivity, specificity, PPV, and NPV of the Aptima CT Assay on the Panther System and the prevalence of *C. trachomatis* in patient-collected vaginal swab and male urine specimens (based on the specimen-specific PIS) by collection site. Prevalence varied across collection sites, as expected. Table 6 shows the PPA and NPA of the assay in female urine specimens by collection site.

Table 5: Performance Characteristics of the Aptima CT Assay in Patient-collected Vaginal Swab and Male Urine Specimens by Collection Site

Specimen Type	Site	N	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
PVS	1	22	8	0	13	1	40.9	88.9 (56.5–98.0)	100 (77.2–100)	100 (NC)	92.9 (78.6–100)
	2	385	12	0	373	0	3.1	100 (75.8–100)	100 (99.0–100)	100 (NC)	100 (NC)
	3	77	5	0	72	0	6.5	100 (56.6–100)	100 (94.9–100)	100 (NC)	100 (NC)
	4	5	1	0	4	0	20.0	100 (20.7–100)	100 (51.0–100)	100 (NC)	100 (NC)
	5	258	26	3	229	0	10.1	100 (87.1–100)	98.7 (96.3–99.6)	89.7 (77.4–100)	100 (NC)
	6	494	50	3	439	2	10.5	96.2 (87.0–98.9)	99.3 (98.0–99.8)	94.3 (87.5–100)	99.5 (98.8–100)
	7	250	42	0	206	2	17.6	95.5 (84.9–98.7)	100 (98.2–100)	100 (NC)	99.0 (97.5–100)
	8	110	5	1	104	0	4.5	100 (56.6–100)	99.0 (94.8–99.8)	83.3 (50.0–100)	100 (NC)
	9	314	8	0	304	2	3.2	80.0 (49.0–94.3)	100 (98.8–100)	100 (NC)	99.3 (98.4–100)
	10	253	17	2	232	2	7.5	89.5 (68.6–97.1)	99.1 (96.9–99.8)	89.5 (72.5–100)	99.1 (97.8–100)
	11	97	2	1	94	0	2.1	100 (34.2–100)	98.9 (94.3–99.8)	66.7 (0.0–100)	100 (NC)
MU	1	177	20	1	156	0	11.3	100 (83.9–100)	99.4 (96.5–99.9)	95.2 (83.3–100)	100 (NC)
	2	373	3	0	370	0	0.8	100 (43.9–100)	100 (99.0–100)	100 (NC)	100 (NC)
	3	61	2	0	59	0	3.3	100 (34.2–100)	100 (93.9–100)	100 (NC)	100 (NC)
	4	13	0	0	13	0	0.0	NC	100 (77.2–100)	NC	100 (NC)
	5	409	30	1	378	0	7.3	100 (88.6–100)	99.7 (98.5–100)	96.8 (88.9–100)	100 (NC)
	6	307	48	2	255	2	16.3	96.0 (86.5–98.9)	99.2 (97.2–99.8)	96.0 (89.7–100)	99.2 (98.0–100)
	7	226	23	0	203	0	10.2	100 (85.7–100)	100 (98.1–100)	100 (NC)	100 (NC)
	8	32	2	0	30	0	6.3	100 (34.2–100)	100 (88.6–100)	100 (NC)	100 (NC)
	9	221	1	1	219	0	0.5	100 (20.7–100)	99.5 (97.5–99.9)	50.0 (0.0–100)	100 (NC)
	10	91	12	0	79	0	13.2	100 (75.8–100)	100 (95.4–100)	100 (NC)	100 (NC)
	11	54	0	0	54	0	0.0	NC	100 (93.4–100)	NC	100 (NC)

CI = confidence interval; **FN** = false negative; **FP** = false positive; **MU** = male urine; **NC** = not calculable; **Prev** = prevalence; **PVS** = patient-collected vaginal swab; **TN** = true negative; **TP** = true positive.

[1]Score CI.

[2]Percentile CI obtained using the bootstrap re-sampling method with 2000 iterations. For some collection sites the statistic is not calculable in some bootstrap samples due to a denominator of zero; the percentile CI is computed using the bootstrap samples in which the statistic can be calculated. If in all bootstrap samples the statistic is not calculable, or if the value of the statistic is constant across all bootstrap samples in which the statistic can be calculated, the 95% percentile bootstrap CI is set to NC.

Table 6: Performance Characteristics of the Aptima CT Assay in Female Urine Specimens by Collection Site

Specimen Type	Site	N	CCA+/ ACT+	CCA-/ ACT+	CCA-/ ACT-	CCA+/ ACT-	PPA % (95% CI)[1]	NPA % (95% CI)[1]
FU	**1**	22	9	1	12	0	100 (70.1–100)	92.3 (66.7–98.6)
	2	385	9	1	375	0	100 (70.1–100)	99.7 (98.5–100)
	3	77	3	0	74	0	100 (43.9–100)	100 (95.1–100)
	4	5	0	0	5	0	NC	100 (56.6–100)
	5	253	19	2	231	1	95.0 (76.4–99.1)	99.1 (96.9–99.8)
	6	484	44	2	436	2	95.7 (85.5–98.8)	99.5 (98.4–99.9)
	7	246	40	1	205	0	100 (91.2–100)	99.5 (97.3–99.9)
	8	111	4	0	107	0	100 (51.0–100)	100 (96.5–100)
	9	260	6	0	254	0	100 (61.0–100)	100 (98.5–100)
	10	251	15	2	234	0	100 (79.6–100)	99.2 (97.0–99.8)
	11	92	2	0	90	0	100 (34.2–100)	100 (95.9–100)

ACT = Aptima Chlamydia trachomatis Assay; **CCA** = composite comparator algorithm; **CI** = confidence interval; **FU** = female urine; **NPA** = negative percent agreement; **PPA** = positive percent agreement.
[1]Score CI.

The performance of the Aptima CT Assay in female urine specimens was also assessed compared to a patient-collected vaginal swab-based CCA, as vaginal swabs are optimal for detection of CT in females. The data showed that the detection of *C*T infection in female urine specimens by the Aptima CT Assay is up to 9.0% lower when using a vaginal swab-based 3-assay CCA.

Chlamydia trachomatis Infected Status Tables

The frequency of test outcomes from comparator NAAT and investigational Panther System testing is summarized in Table 7a, Table 7b, and Table 7c.

Table 7a: C. trachomatis Infected Status for Male Urine Specimens

Specimen Type	Patient Infected Status	NAAT 1	NAAT 2	NAAT 3	ACT Assay	Symptom Status	
						Symptomatic	Asymptomatic
MU	Infected	+	+	N/A	+	83	55
	Infected	+	+	N/A	-	0	1
	Infected	+	-	+	+	1	0
	Infected	-	+	+	+	0	1
	Infected	-	+	+	-	1	0
	Infected	NR	+	+	+	1	0
	Non-infected	-	+	-	+	0	1
	Non-infected	-	+	-	-	3	1
	Non-infected	-	-	N/A	+	4	0
	Non-infected	-	-	N/A	-	702	1046
	Non-infected	-	NR	-	-	2	0
	Non-infected	NR	-	-	-	31	31

ACT Assay = Aptima Chlamydia trachomatis Assay; **MU** = male urine; **N/A** = not applicable; **NR** = no result.

Table 7b: C. trachomatis Infected Status for Patient-collected Vaginal Swab Specimens

Patient Infected Status	NAAT 1		NAAT 2		ACT Assay	Symptom Status	
	PVS	FU	PVS	FU		Symptomatic	Asymptomatic
Infected	+	+	+	+	+	60	72
Infected	+	+	+	+	-	2	2
Infected	+	+	+	-	+	3	1
Infected	+	+	+	NR	+	2	0
Infected	+	-	+	+	+	10	5
Infected	+	-	+	+	-	1	0
Infected	+	-	+	-	+	9	6
Infected	+	NR	+	+	+	0	1
Infected	+	NR	+	+	-	1	0
Infected	+	NR	+	-	-	1	0
Infected	-	+	+	+	+	4	1
Infected	-	+	-	+	+	1	0
Infected	-	+	-	+	-	1	1
Infected	NR	+	+	+	+	0	1
Non-infected	+	-	-	-	+	3	0
Non-infected	+	-	-	-	-	2	6
Non-infected	-	-	+	+	-	0	1
Non-infected	-	-	+	-	+	0	1
Non-infected	-	-	+	-	-	1	1

Table 7b: C. trachomatis Infected Status for Patient-collected Vaginal Swab Specimens

Patient Infected Status	NAAT 1		NAAT 2		ACT Assay	Symptom Status	
	PVS	FU	PVS	FU		Symptomatic	Asymptomatic
Non-infected	-	-	-	+	-	2	0
Non-infected	-	-	-	-	+	3	3
Non-infected	-	-	-	-	-	904	996
Non-infected	-	-	NR	-	-	13	10
Non-infected	-	-	NR	NR	-	0	1
Non-infected	-	NR	-	-	-	35	25
Non-infected	NR	-	-	-	-	3	5
Non-infected	NR	NR	-	-	-	41	24

ACT Assay = Aptima Chlamydia trachomatis Assay; **FU** = female urine; **NR** = no result; **PVS** = patient-collected vaginal swab.

Table 7c: C. trachomatis Composite Comparator Status for Female Urine Specimens

Specimen Type	Composite Comparator Status	NAAT 1	NAAT 2	NAAT 3	ACT Assay	Symptom Status	
						Symptomatic	Asymptomatic
FU	Positive	+	+	N/A	+	66	75
	Positive	+	+	N/A	-	1	0
	Positive	+	NR	+	+	2	0
	Positive	-	+	+	+	4	2
	Positive	-	+	+	-	0	1
	Positive	NR	+	+	+	2	0
	Positive	NR	+	+	-	0	1
	Negative	+	-	-	-	1	1
	Negative	-	+	-	+	3	1
	Negative	-	+	-	-	3	1
	Negative	-	-	N/A	+	4	1
	Negative	-	-	N/A	-	929	1023
	Negative	-	NR	-	-	0	1
	Negative	NR	-	-	-	35	29

ACT Assay = Aptima Chlamydia trachomatis Assay; **FU** = female urine; **NR** = no result; **N/A** = not applicable.

RLU Distribution of Aptima Chlamydia trachomatis Assay Controls

The distribution of the RLU values for the Aptima CT Assay controls is presented in Table 8 from all valid Panther system runs performed during the clinical study.

Table 8: RLU Distribution of Aptima CT Assay Negative and Positive Controls

Control	Statistics	Total RLU (x1000)
Positive Control, CT / Negative Control, GC	N	160
	Minimum	3162
	Median	6816.5
	Maximum	8818
	CV%	7.83
Positive Control, GC / Negative Control, GT	N	160
	Minimum	0
	Median	2.0
	Maximum	30
	CV%	137.49

CV% = percent coefficient of variation; **RLU** = relative light units.
Note: The RLU value reported by the software was the basis for analysis. The reported RLU value is the total measured RLU divided by 1000 with the digits after the decimal point truncated.

Panther System Analytical Performance

Analytical Sensitivity

Analytical sensitivity of the Aptima CT Assay was tested using representative sample matrices. These were urine specimens, vaginal swab specimens, and STM. Panels were made by spiking CT organisms into pools of these matrices at rRNA equivalents of 0.25 IFU/mL and 2.5 IFU/mL (0.5 fg/assay and 5 fg/assay). These panels were tested on three Panther systems using two lots of reagents in replicates of 60 per panel member. Positive agreement with the expected result was calculated. Agreement to expected results was 100% (95% CI 95.7–100%) for all urine panels, for all vaginal swab panels, and for all STM panels. The analytical sensitivity for the Aptima CT Assay is 2.5 IFU/mL.

The limit of detection (LoD) was further tested and confirmed with sensitivity panels prepared using CT serovars E and G spiked into pooled negative vaginal swab matrix. Sensitivity panels were tested on two Panther instruments with two reagent lots. At least 25 replicates were run for each concentration for each reagent lot for each serovar. Each serovar was detected with greater than 95% positivity at less than 2.5 IFU/mL (95% detection at 0.00267 IFU/mL for serovar E and 0.00441 IFU/mL for serovar G).

The analytical sensitivity of the Aptima CT Assay was evaluated for 15 serovars on the DTS System, including serovars E and G, the two serovars giving a similar result on the Panther System.

Analytical Specificity

A total of 154 culture isolates were evaluated using the Aptima CT Assay on the DTS® System. These isolates included 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, parasites and viruses. All organisms except *C. psittaci, C. pneumoniae, U. urealyticum* and the viruses were tested at 1.0×10^6 cells/assay. *C. psittaci* VR601 was tested at 8.0×10^4 cells/assay and *C. psittaci* VR125 was tested at 1.0×10^5 cells/assay. *C. pneumoniae* was tested at 4×10^3 cells/assay and *U. urealyticum* was tested at 6.7×10^6 cells/assay. The viruses were tested as follows: (a) herpes simplex virus I: 2.5×10^4 $TCID_{50}$/assay, (b) herpes simplex virus II: 6.0×10^4 $TCID_{50}$/assay, (c) human papillomavirus 16: 2.9×10^6 DNA copies/assay and (d) cytomegalovirus: 4.8×10^5 cells/assay.

The list of organisms tested is shown in Table 9.

Table 9: Analytical Specificity

Organism	Organism	Organism
Achromobacter xerosis	*Escherichia coli*	*Neisseria mucosa (3)*
Acinetobacter calcoaceticus	*Flavobacterium meningosepticum*	*Neisseria sicca (3)*
Acinetobacter lwoffi	*Fusobacterium nucleatum*	*Neisseria subflava (14)*
Actinomyces israelii	*Gardnerella vaginalis*	*Neisseria perflava*
Actinomyces pyogenes	*Gemella haemolysans*	*Neisseria polysaccharea*
Aerococcus viridans	*Haemophilus ducreyi*	*Paracoccus denitrificans*
Aeromonas hydrophila	*Haemophilus influenzae*	*Peptostreptococcus anaerobius*
Agrobacterium radiobacter	Herpes simplex virus I	*Peptostreptococcus productus*
Alcaligenes faecalis	Herpes simplex virus II	*Plesiomonas shigelloides*
Bacillus subtilis	Human papilloma virus 16	*Propionibacterium acnes*
Bacteriodes fragilis	*Kingella dentrificans*	*Proteus mirabilis*
Bacteriodes ureolyticus	*Kingella kingae*	*Proteus vulgaris*
Bifidobacterium adolescentis	*Klebsiella oxytoca*	*Providencia stuartii*
Bifidobacterium brevi	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Branhamella catarrhalis	*Lactobacillus acidophilus*	*Pseudomonas fluorescens*
Brevibacterium linens	*Lactobacillus brevis*	*Pseudomonas putida*
Campylobacter jejuni	*Lactobacillus jensonii*	*Rahnella aquatilis*
Candida albicans	*Lactobacillus lactis*	*Rhodospirillum rubrum*
Candida glabrata	*Legionella pneumophila (2)*	*Saccharomyces cerevisiae*
Candida parapsilosis	*Leuconostoc paramensenteroides*	*Salmonella minnesota*
Candida tropicalis	*Listeria monocytogenes*	*Salmonella typhimurium*
Chlamydia pneumoniae	*Micrococcus luteus*	*Serratia marcescens*
Chlamydia psittaci (2)	*Moraxella lacunata*	*Staphylococcus saprophyticus*
Chromobacterium violaceum	*Moraxella osloensis*	*Staphylococcus aureus*
Citrobacter freundii	*Morganella morganii*	*Staphylococcus epidermidis*
Clostridium perfringens	*Mycobacterium smegmatis*	*Streptococcus agalactiae*
Corynebacterium genitalium	*Mycoplasma genitalium*	*Streptococcus bovis*
Corynebacterium xerosis	*Mycoplasma hominis*	*Streptococcus mitis*
Cryptococcus neoformans	*N. meningitidis* Serogroup A	*Streptococcus mutans*
Cytomegalovirus	*N. meningitidis* Serogroup B	*Streptococcus pneumoniae*
Deinococcus radiodurans	*N. meningitidis* Serogroup C (4)	*Streptococcus pyogenes*
Derxia gummosa	*N. meningitidis* Serogroup D	*Streptococcus salivarius*
Eikenella corrodens	*N. meningitidis* Serogroup Y	*Streptococcus sanguis*
Enterobacter aerogenes	*N. meningitidis* Serogroup W135	*Streptomyces griseinus*
Enterobacter cloacae	*Neisseria cinerea (4)*	*Trichomonas vaginalis*
Entercoccus avium	*Neisseria dentrificans*	*Ureaplasma urealyticum*
Entercoccus faecalis	*Neisseria elongata (3)*	*Vibrio parahaemolyticus*
Entercoccus faecium	*Neisseria flava*	*Yersinia enterocolitica*
Erwinia herbicola	*Neisseria flavescens (2)*	
Erysipelothrix rhusiopathiae	*Neisseria lactamica (9)*	

(n) = number of strains tested. All organisms tested produced a negative result in the Aptima CT Assay.

Note: *For a nucleic acid amplification assay, analytical specificity with respect to individual organisms is largely determined by the chemistry of the assay (e.g. oligonucleotide sequences), rather than by the platform. Because the reagents for the Aptima CT Assay are identical between the Panther System and the DTS™ systems, analytical specificity for the DTS System is shown in Table 9.*

Interfering Substances

For a nucleic acid amplification assay, analytical specificity with respect to potentially interfering substances is largely determined by the chemistry of the assay (e.g. oligonucleotide sequences) rather than by the platform. Because the reagents for the Aptima CT Assay are identical between DTS and Panther systems, data generated on the DTS System supports the performance of the assay on Panther System.

The following interfering substances were individually spiked into swab, PreservCyt® Solution liquid Pap and/or urine specimens: contraceptive jelly, spermicide, moisturizer, hemorrhoidal anesthetic, body oil, powder, anti-fungal cream, vaginal lubricants, feminine spray and leukocytes (1 x 10^6 cells/mL). The following interfering substances were individually spiked into urine specimens: urine analytes, protein, glucose, ketones, bilirubin, nitrate, urobilinogen, pH 4 (acidic), pH 9 (alkaline), leukocytes (1 x 10^6 cells/mL), cellular debris, vitamins, minerals, acetaminophen, aspirin and ibuprofen. All were tested for potential assay interference in the absence and presence of CT at the estimated rRNA equivalent of 1 cell/assay (5 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. No interference was observed with any of the tested substances. No inhibitors of amplification were observed in the Aptima CT Assay.

Blood commonly found in urogenital specimens may interfere in some amplification assays. Whole blood was used to establish the degree of blood interference on the Panther system with respect to this potential interferant. Fresh blood was added to clinical pools of vaginal swab specimens and urine specimens and then tested for potential assay interference in the presence and absence of CT target. The estimated rRNA equivalent of one (1) CT IFU/assay (5 fg/assay) was used as the target concentration as this represents the analytical sensitivity of the assay. Specimens were tested on the Panther system. All samples containing target nucleic acid were positive when tested at a level of 10% (vol/vol) blood in swab specimens, or 30% (vol/vol) blood in urine specimens. All samples that did not contain target were correctly identified as negative. Blood added to swab and urine specimens at levels much higher than could be expected with normal specimen collection did not interfere with results on the Panther System.

Carryover Studies for the Panther System

A multi-run analytical study was conducted using spiked panels on six Panther systems. Carryover was assessed using approximately 20% high titer CT samples dispersed between negative samples. The runs included clusters of high positive samples with clusters of negative samples as well as single high positives dispersed within the run. High titer samples were made using CT rRNA spiked into STM to give a final concentration of 5 x 10^5 fg rRNA/reaction (rRNA equivalent of 2.5 x 10^5 IFU/mL). Testing was carried out using 5 runs on each of six Panther systems with a total of 5878 negative samples. The overall carryover rate was 0.19% with a 95% confidence interval of 0.10–0.33%.

Reproducibility Study

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

The negative panel member consisted of STM only. The CT positive panel members were created by spiking STM with CT positive cells diluted in STM to achieve the appropriate targeted concentrations (very low positive, low positive, or high positive). Final CT concentrations ranged from 0.25 IFU/mL to 25 IFU/mL.

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

Table 10 shows the signal variability of assay RLU results for each panel member between sites, between operators, between lots, between runs, within runs, and overall (Total). Only samples with valid results were included in the analyses.

Table 10: Reproducibility Study Data-Signal Variability by Panel Member

Panel Member	Target Conc. (IFU/mL)	N	Mean RLU (x1000)	Between Sites		Between Operators		Between Lots		Between Runs		Within Runs		Total	
				SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
Negative	0	107[1]	1.5	0.8	49.7	0.0	0.0	0.0	0.0	0.1	4.9	1.5	101.1	1.7	112.8
Very Low Positive	0.25	108	7339.0	272.0	3.7	0.0	0.0	80.0	1.1	98.2	1.3	142.0	1.9	331.9	4.5
Low Positive	2.5	108	7387.6	307.8	4.2	0.0	0.0	97.9	1.3	139.9	1.9	114.0	1.5	370.0	5.0
High Positive	25	107[1]	7424.4	285.6	3.8	39.6	0.5	136.9	1.8	91.3	1.2	138.7	1.9	359.8	4.8

CV (%) = percent coefficient of variation; **RLU** = relative light units; **SD** = standard deviation.

Notes: The RLU value reported by the software is the total measured RLU divided by 1000 with the digits after the decimal point truncated. Variability from some factors may be numerically negative. In these cases, SD and CV are shown as 0.0.

[1]One invalid result was excluded from the analysis.

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