Aptima™ Chlamydia trachomatis Assay

For *in vitro* diagnostic use.

For U.S. Export only.

DTS™ Systems

Panther™

Tigris™ DTS™

General Information

Intended Use

The Aptima™ Chlamydia trachomatis (CT) assay is a target amplification nucleic acid probe test that utilizes target capture for the in vitro qualitative detection of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) to aid in the diagnosis of chlamydial urogenital disease using the Tigris DTS system or Panther system or using the DTS systems semi-automated instrumentation as specified. The assay may be used to test the following specimens from symptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; and female and male urine specimens. The assay may be used to test the following specimens from asymptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; patient-collected vaginal swab specimens[1]; and female and male urine specimens. This assay is also intended for use with the testing of gynecological specimens, from both symptomatic and asymptomatic patients. These cervical specimens collected in the PreservCyt™ Solution vials may be tested either pre- or post-Pap processing. Testing of post-Pap processed specimens is limited to specimens processed with the ThinPrep™ 2000 System only.

[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 is not 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 1,808,703 cases of CT infections (553 cases per 100,000 population) were reported to the Centers for Disease Control in 2019 (5).

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 (29). The serovars D through K are the major cause of genital chlamydial infections in men and women (21). *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and pelvic inflammatory disease (3, 13, 23, 24). *C. trachomatis* infections are often asymptomatic in both males and females. Children born to infected mothers are at significantly higher risk for inclusion conjunctivitis and chlamydial pneumonia (1, 10, 22).

Historically, several methods for CT detection have been utilized in the clinical laboratory, including cell culture, direct fluorescent antibody testing, and enzyme immunoassay. More recent methodologies for CT detection include direct DNA probe assays and nucleic acid amplification tests (NAATs). Cell culture was once considered to be the “gold standard” for detection of CT. Culture is quite specific, but recent publications have demonstrated that NAATs have a higher clinical sensitivity than culture (2, 8, 14, 25). Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe and NAATs.

First generation NAATs for CT have technological issues that have limited their performance. These issues include cumbersome specimen processing and specimen inhibition that can yield false negative results (6, 12, 15, 20, 26, 28). The Aptima CT assay is a second generation 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 target capture, TMA, and HPA (7, 11).

According to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* 2002 Screening Guidelines, CDC recommends a number of options for follow-up on a positive screening test "if a low positive predictive value can be expected or if a false-positive result would have serious psychosocial or legal consequences" (4). One of these options for additional testing can be a different FDA-cleared nucleic acid amplification test that targets a different nucleic acid sequence than the initial test. 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 combines the technologies of target capture, TMA, and HPA.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the Aptima CT assay is performed in the laboratory, the target rRNA molecule is isolated from the specimens by use of 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 hybridization. A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU).

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. 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.

D. For additional specific warnings, precautions and procedures to control contamination for the Tigris DTS system, consult the *Tigris DTS System Operator's Manual*.

E. For additional specific warnings, precautions and procedures to control contamination for the Panther/Panther Fusion system, consult the *Panther System Operator's Manual*.

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

J. 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.

DTS Systems Specific

K. A separate area for HPA is strongly recommended to minimize amplicon contamination in the assay. This dedicated area should be away from the reagent preparation, target capture, and amplification areas.

L. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a unidirectional workflow: from reagent preparation through HPA. Specimens, equipment, and reagents should not be returned to the area where a previous step was performed. Also, personnel should not move back into previous work areas without observing proper contamination safeguards.

Specimen Related

M. This assay has been tested using endocervical and male urethral swab specimens, PreservCyt solution liquid Pap specimens, vaginal swab specimens, female and male urine specimens only. Performance with specimens other than those specified under Specimen Collection and Storage has not been evaluated.

Laboratories may validate other collection devices (16, 18).

N. 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.

O. The PreservCyt solution has been validated as an alternative medium for testing with the Aptima CT assay. PreservCyt solution liquid Pap specimens processed using instruments other than the ThinPrep 2000 processor have not been evaluated for use in Aptima assays.

P. After urine has been added in the urine transport tube, the liquid level must fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.

Q. 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.

R. 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.

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

T. If the lab receives a swab specimen transport tube with no swab, two swabs, a cleaning swab, or a swab not supplied by Hologic, the specimen must be rejected. Prior to rejecting a swab transport tube with no swab, verify that it is not an Aptima™ Specimen Transfer Tube as this specimen transport tube will not contain a swab.

U. For PreservCyt solution liquid Pap specimens, collect according to the manufacturer's instructions. Aliquots subsequently removed from the PreservCyt vial for testing by the Aptima CT assay should be processed using only the Aptima Specimen Transfer Kit.

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

Assay Related

W. The performance of vaginal swab specimens has not been evaluated in pregnant women.

X. The performance of endocervical, vaginal, and male urethral swab specimens, male and female urine specimens, and PreservCyt solution liquid Pap specimens has not been evaluated in adolescents less than 16 years of age.

Y. Do not use this kit after its expiration date.

Z. 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* for more information.

AA. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids.

AB. Avoid microbial and ribonuclease contamination of reagents.

AC. **Do not interchange, mix, or combine assay reagents** from kits with different lot numbers. Aptima controls and assay fluids can be from different lot numbers.

DTS Systems Specific

AD. Tips with hydrophobic plugs must be used. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the target capture and amplification steps, and one for use in the HPA steps. Two micropipettors must be dedicated for use in this assay: one for use in specimen transfer and one for use in reagent preparation. All pipettors must be cleaned regularly as described in *DTS Systems Test Procedure*, *Procedural Notes*.

AE. When using repeat pipettors for reagent addition, do not touch the tube with the pipette tip to prevent carryover from one tube to another.

AF. Adequate mixing is necessary to achieve accurate assay results. For complete details, see *DTS Systems Test Procedure*, *Procedural Notes*.

AG. Separate water baths must be dedicated for the target capture, amplification, and HPA steps in the assay.

AH. Assay reproducibility was established using spiked Specimen Transport Media (STM) with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined.

AI. Sealing cards should be disposed of in the waste container immediately after removing them from reaction tubes. Fresh sealing cards should always be used: they should never be re-used from a previous step. Sealing cards should be firmly fixed to the top of all reaction tubes.

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

Note: *Hazard communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on 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.*

EU Hazard Information	
—	**Amplification Reagent** *HEPES 25 – 30%* — H412 – Harmful to aquatic life with long lasting effects P273 – Avoid release to the environment P280 – Wear eye protection/ face protection
—	**Enzyme Reagent** *HEPES 1 – 5%* — H412 – Harmful to aquatic life with long lasting effects P273 – Avoid release to the environment P280 – Wear eye protection/ face protection

—	**Probe Reagent** *LAURYL SULFATE LITHIUM SALT 35 - 40%* *SUCCINIC ACID 10 - 15%* *LITHIUM HYDROXIDE, MONOHYDRATE 10 - 15%* **—** H412 - Harmful to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/ face protection
	**Selection Reagent** *BORIC ACID 1 - 5%* **WARNING** H315 - Causes skin irritation
—	**Target Capture Reagent** *HEPES 5 – 10%* *EDTA 1 – 5%* *LITHIUM HYDROXIDE, MONOHYDRATE 1 – 5%* **—** H412 – Harmful to aquatic life with long lasting effects P273 – Avoid release to the environment P280 – Wear eye protection/ face protection
	Plus These for DTS Systems
 	**Buffer for Deactivation Fluid** *SODIUM HYDROXIDE 1 - 5%* *SODIUM HYPOCHLORITE <1%* **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation H411 - Toxic to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/ face protection
	**Aptima Oil** *POLYDIMETHYLSILOXANE 100%* **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation

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	2ºC to 30ºC	2ºC to 8ºC	60 days
Selection Reagent	2ºC to 30ºC	2ºC to 30ºC	N/A
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. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Target Capture Reagent

Wash Solution

Buffer for Deactivation Fluid

Oil Reagent

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

H. Reagents stored on-board the Panther system have 72 hours of on-board stability.

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 CT in clinician-collected endocervical, vaginal and male urethral swab specimens, patient-collected vaginal swab specimens, female and male urine specimens, and PreservCyt solution liquid Pap specimens. Performance with specimens other than those collected with the following specimen collection kits has not been evaluated:

- Aptima Mulititest Swab Specimen Collection Kit
- Aptima Urine Collection Kit for Male and Female Urine Specimens
- Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- Aptima Specimen Transfer Kit (for use with gynecologic samples collected in PreservCyt solution)

A. Instructions for Collection

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

B. Specimen Transport and Storage Before Testing

1. Urogenital Swab Specimens
   a. After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested. Specimens must be assayed with the Aptima CT assay within 60 days of collection. If longer storage is needed, freeze urogenital specimens in the swab specimen transport tube within 7 days of collection at –20°C to –70°C to allow testing for up to 12 months after collection (see Specimen Stability Studies).
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 for up to 12 months after collection (see Specimen Stability Studies).
3. PreservCyt Solution Liquid Pap Specimens
   a. PreservCyt Solution liquid Pap specimens intended for CT testing must be processed for cytology and/or transferred to an Aptima specimen transfer tube within 30 days of collection when stored at 2°C to 30°C (see Specimen Stability Studies).
   b. If the ThinPrep aliquot removal procedure will be used, refer to the *ThinPrep Systems Processor Operator's Manual* for instructions on aliquot removal. Transfer 1 mL of the removed aliquot into an Aptima specimen transfer tube according to the instructions in the Aptima Specimen Transfer Kit package insert.

c. If testing the specimen after processing using the ThinPrep systems processor, process the PreservCyt solution liquid Pap specimen in accordance with the *ThinPrep Systems Processor Operator's Manual* and the Aptima Specimen Transfer Kit package insert. Transfer 1.0 mL of the fluid remaining in the PreservCyt solution vial into an Aptima specimen transfer tube according to the instructions in the Aptima Specimen Transfer Kit package insert.

d. Once the PreservCyt solution liquid Pap specimen is transferred to the Aptima specimen transfer tube, the specimen must be assayed with the Aptima CT assay within 30 days when stored at 2°C to 8°C or 14 days when stored at 15°C to 30°C. If longer storage is needed, freeze specimen within 7 days of transfer to the Aptima specimen transfer tube at –20°C to –70°C to allow testing up to 12 months after transfer (see Specimen Stability Studies).

C. Specimen Storage After Testing

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen transport tubes should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable caps and place new non-penetrable or 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.**

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

DTS Systems

Reagents for the Aptima CT assay are listed below for the DTS systems. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima Chlamydia trachomatis Assay Kit, 100 tests (2 boxes) (Cat. No. 301088)

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 buffered solution containing < 5% detergent.*	1 x 0.35 mL
PCT/NGC	**Positive Control, CT / Negative Control, GC** *Non-infectious CT nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 1 CT IFU (5 fg/assay*).*	3 x 1.7 mL
PGC/NCT	**Positive Control, GC / Negative Control, CT** *Non-infectious GC nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 50 GC cells (250 fg/assay*).*	3 x 1.7 mL

*The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism.

Also included in the refrigerated box are the following (Storage Tray)
(store at 2°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	**Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 9.3 mL
ER	**Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 3.3 mL
PR	**Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 12.4 mL
S	**Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 31 mL
	Reconstitution Collars	3
	Sealing Cards	1 package

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

Symbol	Component	Quantity
TCR	**Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 22 mL
W	**Wash Solution** *10 mM HEPES buffered solution containing < 2% detergent.*	1 x 402 mL
DF	**Buffer for Deactivation Fluid** *800 mM bicarbonate buffered solution.*	1 x 402 mL
O	**Oil Reagent** *Silicone Oil*	1 x 24.6 mL

Materials Required But Available Separately

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

	Cat. No.
Leader HC+ Luminometer	104747-01
Hologic Target Capture System (TCS)	104555
Incubators and vortexers:	
2 Multi-tube vortex mixers *102160G*	
3 Circulating water baths *104586* *(62ºC ± 1ºC, 42ºC ± 1ºC, 62ºC ± 1ºC)*	
3 Water bath spacers *104627*	
OR	
2 SB100 Dry Heat Bath/Vortexers *105524*	
Additional SB100 baths may be required as test volume increases	
Aptima Auto Detect Kit	301048
2 eppendorf Repeater Plus pipettors	MME-02362
2 pipettors, 1000 µL RAININ PR1000	901715
eppendorf pipettor, 20 µL to 200 µL	105726
Repeat pipettor tips, 2.5 mL	21-381-329
Repeat pipettor tips, 5.0 mL	21-381-330
Repeat pipettor tips, 25.0 mL	21-381-115
Tips, P1000 Style *special diameter tip only available from Hologic*	105049
Pipette tips 20 µL to 200 µL	705512 (Fisher)
Ten Tube Units (TTU)	TU0022
Ten Tip Cassettes (TTC)	104578

	Cat. No.
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Aptima Urine Specimen Collection Kit for Male and Female Urine Specimens	301040
Aptima Urine Specimen Transport Tubes	105575
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit — printable	PRD-05110
SysCheck calibration standard	301078
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Standard urine collection containers, without preservatives	—
Large-capped plastic container	—
Aptima penetrable caps	105668
Replacement non-penetrable caps	103036A

Optional Materials

		Cat. No.
Aptima Controls Kit		301110
Aptima Assay Fluids *Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent*		302002C
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*		302101
Tips, 1000 µL conductive, liquid sensing		10612513 (Tecan)
TECAN Freedom EVO 100/4 containing		900932
DTS 800 Systems Aptima Combo 2 Deck Plate	*105200*	
Reagent reservoir (40 mL quarter module)	*104765*	
Split reagent reservoir (19 mL x 2 quarter module)	*104763*	
Tube Rocker		—
Lint-free Wipes		—
Plastic-backed Bench Covers		—

DTS Systems Test Procedure

A. Equipment Preparation

1. Adjust one water bath to 62°C ± 1°C (for target capture, and primer annealing), a second water bath to 42°C ± 1°C (for amplification), and a third water bath to 62°C ± 1°C (for HPA). If using the SB100™ Dry Heat Bath/Vortexer, refer to the *SB100 Dry Heat Bath/Vortexer Application Sheet* (*SB100 Application Sheet*).

2. Prior to starting the assay, wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces and pipettors for at least one minute, then follow with a deionized water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed, absorbent laboratory bench covers.
3. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with Wash Solution and the aspiration manifold is connected to the vacuum pump. (Refer to the *Target Capture System Operator's Manual*.)

B. Reagent Reconstitution

Note: *Reagent reconstitution should be performed prior to beginning specimen transfer.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair the appropriate reconstitution solution with the lyophilized reagent. The labels are color coded so that they can be paired correctly.
   b. 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).
   c. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
   d. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 1, Step 2).
   e. Slowly invert the assembled bottle and vial. Allow the solution to drain from the reconstitution solution bottle into the vial (Figure 1, Step 3).
   f. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 1, Step 4).
   g. Wait for the lyophilized reagent to go into solution, then invert the assembled bottle and vial 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.
   h. Remove the reconstitution collar from the bottle (Figure 1, Step 6).
   i. Recap the reconstitution solution bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).
   j. Discard the reconstitution collar and vial (Figure 1, Step 8).

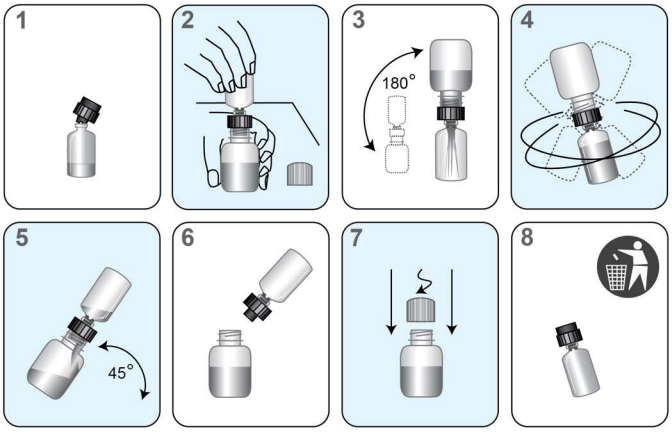

Figure 1. DTS Systems Reconstitution Process

2. Previously reconstituted Probe, Amplification, and Enzyme Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay. If Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. After resuspension, mix by gentle inversion, being careful not to induce foam.

Note: *This inversion step should be performed any time that the precipitate is being brought into solution, whether by heating at 62°C or by warming at room temperature.*

3. Prepare Working Target Capture Reagent (wTCR)
   a. Transfer 20 mL of TCR to an appropriately sized, dedicated, clean, dry container.
   b. Using a micropipettor, add 200 µL of TCR-B into the TCR.
   c. Thoroughly mix the solution by swirling.
   d. Label the container. Record operator initials, preparation date, and both lot numbers.

Note: *For a smaller number of reactions (specimens and controls), use the following to calculate volumes of TCR and TCR-B:*

Volume of TCR (mL) = (number of reactions + 5 extra reactions) x 0.1 mL

Volume of TCR-B (mL) = Volume of TCR (mL) / 100

C. Target Capture

The repeat pipettor used in target capture and amplification should be dedicated for use in these steps only. See *Warnings and Precautions* for more information.

Rack Setup

1. Allow the controls and specimens to reach room temperature prior to processing.
2. Do not vortex specimens.
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single blue Aptima collection swab in a unisex swab specimen transport tube.

b. The presence of a single pink Aptima collection swab in a multitest or vaginal swab specimen transport tube.

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

d. The absence of a swab in the Aptima specimen transport tube for PreservCyt solution liquid Pap specimens.

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

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

5. If specimens with standard caps (non-penetrable caps) are to be tested, they 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 before uncapping. **Avoid splashing and cross-contamination.**
6. In the Ten Tube Unit (TTU) rack, place enough TTUs to accommodate the controls and specimens.
7. If a worklist is desired, create the worklist at this point. For instructions on creating a worklist, refer to the *Aptima Assay Software Operator's Manual*.
8. Thoroughly mix the wTCR. Using the repeat pipettor, add 100 µL into each reaction tube.
9. **The first reaction tube of the assay must contain the negative control, and the second reaction tube must contain the positive control.**
   a. The negative control label for the Aptima CT assay is blue-green. The label text identifies the negative control as "CONTROL + GC PGC / CONTROL – CT NCT". The positive control label for the Aptima CT assay is pink. The label text identifies the positive control as "CONTROL + CT PCT / CONTROL – GC NGC".
   b. Hold the negative control tube (blue-green-labeled tube) in one hand or keep it in a rack. Using a micropipettor, pierce the cap, taking care not to drive the tip into the bottom of the tube. Add 400 µL of the negative control (blue-green-labeled tube) to the first reaction tube. In the same manner and using a new pipette tip, add 400 µL of the positive control (pink-labeled tube) to the second reaction tube.
10. Continue the rack setup procedure by adding 400 µL of each specimen into the remaining reaction tubes. Use a new pipette tip for each specimen and control. The acceptable volume of specimen or control added to a reaction tube is 400 µL ± 100 µL. See *Procedural Notes*, *Control and Specimen Pipetting* for more information.

Target Capture

Use of the Hologic™ Target Capture System is described in the *Target Capture System Operator's Manual*. If using the SB100 Dry Heat Bath/Vortexer, refer to the *SB100 Application Sheet*.

11. Cover the TTUs with sealing cards and shake the rack gently by hand. **Do not vortex.** Incubate the rack at 62°C ± 1°C in a water bath for 30 ± 5 minutes.
12. Remove the rack from the water bath and blot the bottoms of the tubes dry on absorbent material.
13. Ensure the sealing cards are firmly seated. If necessary, replace them with new sealing cards and seal the TTUs tightly.
14. Vortex the rack for 60 seconds on the multi-tube vortex mixer. See *Procedural Notes, Vortexing* for details. Begin vortexing within 2 minutes of removal of the rack from the water bath.
15. Without removing the sealing cards, incubate the rack at room temperature for 30 ± 5 minutes.
16. Place the rack on the TCS magnetic base for 5 to 10 minutes.
17. Prime the dispense station pump line by pumping Wash Solution through the dispense manifold. Pump enough liquid through the system so that there are no air bubbles in the line and that all ten nozzles are delivering a steady stream of liquid.
18. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the aspiration manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification.[2] It may take 15 seconds to achieve this reading. Reconnect the aspiration manifold, and ensure that the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed and the aspiration manifold tubing is dry.
19. Firmly attach the aspiration manifold to the first set of tips. Aspirate all liquid by lowering the tips into the first TTU until the tips come into brief contact with the bottoms of the tubes. Do not hold the tips in contact with the bottoms of the tubes.
20. After the aspiration is complete, eject the tips into their original TTC. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each specimen.
21. Place the dispense manifold over each TTU and, using the dispense station pump, deliver 1.0 mL of Wash Solution into each tube of the TTU.
22. Cover the tubes with a sealing card and remove the rack from the TCS magnetic base. Vortex the rack once on the multi-tube vortex mixer. See *Procedural Notes, Vortexing* for details.
23. Place the rack on the TCS magnetic base for 5 to 10 minutes.
24. Aspirate all liquid as in Steps 19 and 20.
25. After the final aspiration, remove the rack from the TCS magnetic base and visually inspect the tubes to ensure that all liquid has been aspirated, and all tubes contain magnetic particle pellets. If any liquid is visible, place the rack back onto the TCS magnetic base for 2 minutes and repeat the aspiration for that TTU using the same tips used previously for each specimen.

Note: *If a magnetic particle pellet is visible after aspiration is completed, the tube may be accepted. If no pellet is visible, the specimen should be retested. If the same specimen does not contain a magnetic particle pellet at this step in a subsequent run, this may indicate a specimen-specific problem. Re-collection of the specimen is recommended in this situation.*

2 See the Target Capture System Vacuum Specifications Sheet located at the back of the *Target Capture System Operator's Manual* or contact Technical Support.

D. Amplification

If using the SB100 Dry Heat Bath/Vortexer, refer to the *SB100 Application Sheet*.

1. Using the repeat pipettor, add 75 µL of the reconstituted Amplification Reagent CT to each reaction tube. All reaction mixtures in the rack should now be red.
2. Using the repeat pipettor, add 200 µL of Oil Reagent to each reaction tube.
3. Cover the tubes with a sealing card and vortex them on the multi-tube vortex mixer.
4. Incubate the rack in a water bath at 62°C ± 1°C for 10 ± 5 minutes.
5. Transfer the rack into a water bath at 42°C ± 1°C and incubate for 5 ± 2 minutes.
6. With the rack in the water bath, carefully remove the sealing card and, using the repeat pipettor, add 25 µL of the reconstituted Enzyme Reagent to each reaction tube. All reaction mixtures should now be orange.
7. Immediately cover the tubes with a fresh sealing card, remove the rack from the water bath, and mix the reaction tubes by gently shaking the rack by hand.
8. Incubate the rack in a water bath at 42°C ± 1°C for 60 ± 15 minutes.

E. Hybridization Protection Assay (HPA)

If using the SB100 Dry Heat Bath/Vortexer, refer to the *SB100 Application Sheet*.

The repeat pipettor used in the hybridization and selection steps should be dedicated for use in these steps only. See *Warnings and Precautions*.

1. Hybridization
   a. Remove the rack from the water bath and transfer it to the HPA area. Using the repeat pipettor, add 100 µL of the reconstituted Probe Reagent to each reaction tube. All reaction mixtures should now be yellow.
   b. Cover the tubes with a sealing card and vortex the rack on the multi-tube vortex mixer.
   c. Incubate the rack in a 62°C ± 1°C water bath for 20 ± 5 minutes.
   d. Remove the rack from the water bath and incubate it at room temperature for 5 ± 1 minutes.
2. Selection
   a. Using the repeat pipettor, add 250 µL of Selection Reagent to each reaction tube. All reaction mixtures should now be red.
   b. Cover the tubes with a sealing card, vortex the rack for 10 seconds or until the color is uniform, and incubate the rack in a water bath at 62°C ± 1°C for 10 ± 1 minutes.
   c. Remove the rack from the water bath.
3. Detection

   Detection must be performed at 18°C to 28°C.

   a. Incubate the rack at 18°C to 28°C for 15 ± 3 minutes.

Note: *This temperature range is critical for assay performance.*

   b. For use of the Leader™ HC+ Luminometer and the Aptima Assay software, refer to the *Leader HC+ Luminometer Operator's Manual* and the *Aptima Assay Software Operator's Manual.*
   c. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the tests.

d. Prepare the Leader HC+ Luminometer by placing one empty TTU in cassette position number 1 and performing the **Wash** protocol.

e. Load the TTUs into the luminometer.

f. Log on to the computer. Click on **New Run,** choose **Aptima CT Assay Protocol** and enter the number of tubes (controls and specimens). Click **Next** to begin the run.

Note: *The run must be completed within 2 hours of the end of the selection step incubation.*

g. Prepare Deactivation Fluid by mixing equal volumes of 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution and Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. Deactivation Fluid is stable for 4 weeks at room temperature. Discard Deactivation Fluid after 4 weeks or after 100 processed samples have been deactivated (whichever comes first).

h. After removing the used TTUs from the luminometer, place the TTUs into the container of Deactivation Fluid. Allow the TTUs to sit in the container for 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

Procedural Notes

A. Controls

To work properly with the Aptima Assay software, the Negative Control for CT, which is labeled “CONTROL + GC PGC / CONTROL – CT NCT,” must be in the first position of the first TTU. The Positive Control for CT, which is labeled “CONTROL + CT PCT / CONTROL – GC NGC,” must be in the second position of the first TTU. Placement in the wrong position will cause the run to fail. Any additional controls must be entered as patient specimens and monitored by the operator for acceptability. The Positive Control for GC serves as the Negative Control for the Aptima CT assay.

B. Control and Specimen Pipetting

The volume of control or specimen added to the reaction tube should be 400 µL ± 100 µL. Visual inspection of the volume pipetted into the reaction tube is recommended to ensure proper volume transfer. Proper control or specimen volume is needed to provide accurate results. If the proper volume has not been pipetted, re-pipette the wTCR CT and the control or specimen into a new reaction tube.

C. Reagents

Probe Reconstitution Solution may precipitate during storage. If this occurs, heat the Probe Reconstitution Solution at 62°C for 1 to 2 minutes. After this heat step, the Probe Reconstitution Solution may be used even if residual precipitate remains. After resuspension, mix the vial by gentle inversion, being careful not to induce foam.

D. Temperature

1. The target capture, amplification, hybridization, and selection steps are temperature dependent. Therefore, it is imperative that the water baths be maintained within their specified temperature ranges.
2. Room temperature is defined as 15°C to 30°C.
3. The detection steps in the assay must be carried out at 18°C to 28°C.

E. Time

The target capture, amplification, hybridization, and selection reactions are all time dependent. Adhere to the times specified in the *DTS Systems Test Procedure*.

F. Vortexing

Proper vortexing is important to the successful performance of the Aptima CT assay. When adequate vortexing motion is achieved, the suspension rotates at a rate that raises the solution into the upper half of the tube. This manipulation (vortexing) is maintained for specified periods of time. To vortex reactions, set the multi-tube vortex mixer speed to the lowest setting, secure the rack, and turn on power. Slowly increase speed until the liquid rises halfway up the tube. Vortex for 10 seconds, the indicated amount of time, or until the color is uniform. Then, turn speed to lowest setting before turning off the multi-tube vortex mixer and removing the rack. The reaction mixtures should never touch the sealing cards.

G. Water Baths

1. The level of the water in the water baths must be maintained at 1.5 inches to 2.0 inches (3.8 cm to 5 cm) deep as measured from the supporting metal tray (on the bottom of the water bath) to the surface of the water. This will ensure proper heat transfer.
2. To avoid cross-contamination, water baths should be dedicated to a specific assay step.

H. Decontamination

1. Surfaces and Pipettors

   Laboratory bench surfaces and pipettors must be decontaminated regularly with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Chlorine solutions may pit equipment and metal. Thoroughly rinse equipment with water to avoid pitting.

2. TCS Aspiration Manifold
   a. Place a new TTC into the TTC rack. Turn on the vacuum pump. Attach the aspiration manifold to the tips in the TTC. Aspirate all Wash Solution remaining in the priming trough of the Wash Solution dispense station. (Move the dispense manifold out of the way.)
   b. Pour at least 100 mL of 0.5% to 0.7% (0.07 M to 0.1 M), or if preferred 2.5% to 3.5% (0.35 M to 0.5 M), sodium hypochlorite solution into the priming trough. Aspirate all of the solution through the aspiration manifold.
   c. Pour at least 100 mL of deionized water into the priming trough. Aspirate all of the water through the aspiration manifold.
   d. Eject the tips into their original TTC.
   e. Leave the vacuum pump on until the manifold tubing is dry to prevent back flow.
   f. Decontaminate the aspiration manifold surfaces as described in *TCS Unit*.
3. TCS Waste Container

   When the waste bottle is 25% full or weekly, remove the waste bottle from the Target Capture System.

   a. Turn off the vacuum pump and allow the vacuum pressure to equalize.

b. Release the quick disconnect fittings between the waste bottle and overflow bottle, and the waste bottle and aspiration manifold.

c. Remove the waste bottle from the vacuum trap enclosure.

d. Remove the cap and carefully add 400 mL of 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution to the bottle (or 1 L if using a 10 L waste bottle).

Note: *This may be done in a fume hood to avoid the release of fumes into the laboratory.*

e. Cap the waste bottle and gently swirl the contents until fully mixed.

f. Let the waste bottle sit for 15 minutes and then dispose of the contents (waste).

g. Rinse the waste bottle with water to remove any remaining waste.

h. Cap the empty waste bottle and place it in the vacuum trap enclosure. Attach the quick disconnect fitting to the TCS unit. Carefully discard both gloves.

4. TCS Unit

Wipe the surfaces of the TCS unit, aspiration manifold, and wash buffer ejector tips with paper towels moistened with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Follow the sodium hypochlorite step with a water rinse and then dry the surfaces completely with paper towels.

5. Racks

Submerge the racks in 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution, ensuring that they are covered by the sodium hypochlorite solution. Keep the racks submerged for 10 minutes. Longer exposure could damage the racks. Rinse the racks thoroughly with water, place the racks on a clean absorbent pad, and allow the racks to air-dry thoroughly. To prolong the life of the racks, allow the racks to dry upright, not upside-down.

I. Assay Contamination

1. The introduction of contaminating materials may occur if sufficient care is not taken during the assay protocol.
2. TTUs must be decontaminated in Deactivation Fluid as described under *Detection*. Do not reuse the TTUs.
3. Perform regular decontamination of equipment and work surfaces as described in *Procedural Notes*, *Decontamination*.
4. As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

J. Lab Contamination Monitoring Protocol for DTS Systems

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

To monitor for laboratory contamination, the following procedure may be performed using the Aptima unisex swab specimen collection kit for endocervical and male urethral swab specimens.

1. Label swab transport tubes with numbers corresponding to the areas to be tested.

2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the STM, and swab the designated area using a circular motion.
3. Immediately insert the swab into a 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 the swab using the Aptima CT assay according to the *DTS Systems Test Procedure*.

If the results are CT positive or equivocal (see *Test Interpretation — QC/Patient Results*), the surface may be contaminated and should be decontaminated by treating with sodium hypochlorite solution as recommended in *DTS Systems Test Procedure, Equipment Preparation*.

Note: *If contamination of the water bath is suspected, the bath water can be tested using the urine specimen test procedure, by adding 2.0 mL of the water to a urine specimen transport tube.*

K. Troubleshooting

1. Low positive control values may be caused by incorrect temperatures during various steps in the assay or by allowing the selection time in the selection step to go longer than the recommended time.
2. High backgrounds may occur if the selection time in the selection step is shortened, the selection temperature is not correct, or insufficient mixing occurs after the addition of the Selection Reagent.
3. If the positive control for GC, which is labeled “CONTROL + GC PGC / CONTROL – CT NCT”, is positive or equivocal for CT, see *Procedural Notes, Assay Contamination* for more information.

Tigris DTS System

Reagents for the Aptima CT assay are listed below for the Tigris DTS 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. 303091)

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. Each 400 µL sample contains the estimated rRNA equivalent of 1 CT IFU (5 fg/assay*).*	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. Each 400 µL sample contains the estimated rRNA equivalent of 50 GC cells (250 fg/assay*).*	5 x 1.7 mL

*The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism.

Materials Required But Available Separately

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

		Cat. No.
Tigris DTS System		105118
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*		302382
Aptima Auto Detect Kit		301048
Aptima System Fluid Preservative Kit		302380
Tips, 1000 µL, 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
Tigris DTS System Run Kit containing		301191
Multi-tube Units (MTU)	*104772-02*	
MTU-Tiplet Waste Bag Kit	*900907*	
MTU Waste Deflectors	*900931*	
MTU Waste Covers	*105523*	
Aptima Specimen Transfer Kit *for use with specimens in PreservCyt Solution*		301154C
Aptima Specimen Transfer Kit — printable *for use with specimens in PreservCyt Solution*		PRD-05110
Aptima Multitest Swab Specimen Collection Kit		PRD-03546
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens		301041
Aptima Urine Specimen Collection Kit for Male and Female Urine Specimens		301040
Aptima Urine Specimen Transport Tubes		105575
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution		—
Water for the Tigris DTS System *consult the Tigris DTS System Operator's Manual for specifications*		—
Disposable gloves		—
SysCheck calibration standard		301078
Aptima penetrable caps		105668
Replacement non-penetrable caps		103036A
Replacement caps for 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

Tigris DTS System Test Procedure

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

A. Work Area Preparation

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

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent reconstitution should be performed prior to beginning any work on the Tigris DTS system.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 2, Step 1).
   d. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 2, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the reconstitution solution bottle into the glass vial (Figure 2, Step 3).
   g. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 2, Step 4).
   h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 2, Step 5). Allow all of the liquid to drain back into the reconstitution solution bottle.
   i. Remove the reconstitution collar and glass vial (Figure 2, Step 6).

j. Recap the bottle.
   - For 100-test bottles, record operator initials and the reconstitution date directly on the label (see Figure 3).

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

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

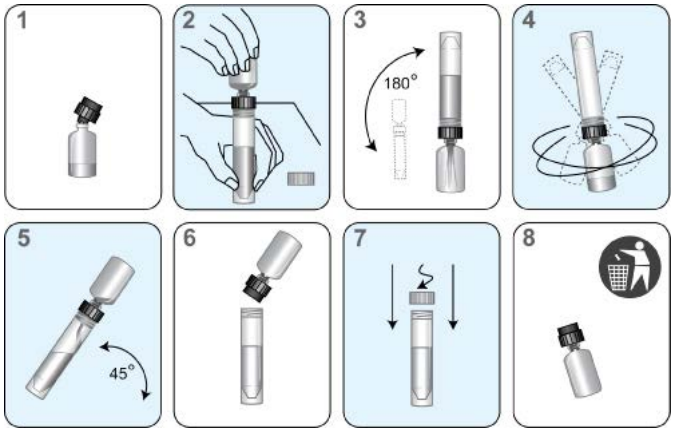

Figure 2. Tigris DTS System Reconstitution Process

2. Prepare working TCR (wTCR) for the 100-test kit
   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.
2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.

3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.
4. Do not top off reagent bottles. The Tigris DTS system will recognize and reject bottles that have been topped off.

D. Specimen Handling

1. Allow the controls and specimens to reach room temperature prior to processing.
2. Do not vortex specimens.
3. Visually confirm that each specimen tube meets one of the following criteria.
   a. The presence of a single blue Aptima collection swab in a unisex swab specimen transport tube.
   b. The presence of a single pink Aptima collection swab in a multitest or vaginal swab specimen transport tube.
   c. A final volume of urine between the black fill lines of a urine specimen transport tube.
   d. The absence of a swab in the Aptima specimen transport tube for PreservCyt solution liquid Pap specimens.
4. Inspect specimen tubes before loading into rack.
   a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.
   c. If the liquid level in a urine specimen tube is not between the two black indicator lines on the label, the specimen must be rejected. Do not pierce an overfilled tube.
   d. If a urine specimen tube contains precipitate, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, visually ensure that the precipitate does not prevent delivery of the specimen.

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

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

E. System Preparation

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

Procedural Notes

A. Controls

1. To work properly with the Tigris Aptima Assay software, front and end controls are required. The Positive Control, GC / Negative Control, CT must be in the first position and second to last position of a worklist. This control label is blue-green. The label text is “CONTROL + GC PGC / CONTROL – CT NCT”. The Positive Control, CT / Negative Control, GC must be in the second position and last position of a worklist. This control label is pink. The label text is “CONTROL + CT PCT / CONTROL – GC NGC”.
2. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to insufficient volume errors.

B. Temperature

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

C. 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 Tigris DTS System

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

To monitor for laboratory contamination, the following procedure may be performed using the Aptima unisex swab specimen collection kit for endocervical and male urethral swab specimens.

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

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

Panther System

Reagents for the Aptima 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. Each 400 µL sample contains the estimated rRNA equivalent of 1 CT IFU (5 fg/assay*).*	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. Each 400 µL sample contains the estimated rRNA equivalent of 50 GC cells (250 fg/assay*).*	5 x 1.7 mL

*The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism.

Materials Required But Available Separately

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

	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL, 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 Specimen Transfer Kit *for use with specimens in PreservCyt Solution*	301154C
Aptima Specimen Transfer Kit — printable *for use with specimens in PreservCyt Solution*	PRD-05110
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Aptima Urine Specimen Collection Kit for Male and Female Urine Specimens	301040
Aptima Urine Specimen Transport Tubes	105575
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable gloves	—
SysCheck calibration standard	301078

Aptima penetrable caps		105668
Replacement non-penetrable caps		103036A
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	—
Lint-free Wipes	—
Plastic-backed Bench Covers	—

Panther System Test Procedure

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

A. Work Area Preparation

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

B. Reagent Reconstitution/Preparation of a New Kit

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

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 3, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 3, Step 2).

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

g. Thoroughly mix the solution in the glass vial by swirling (Figure 3, Step 4).

h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 3, Step 5). Allow all of the liquid to drain back into the reconstitution solution bottle.

i. Remove the reconstitution collar and glass vial (Figure 3, Step 6).

j. Recap the plastic bottle. Record operator initials and the reconstitution date on the label (Figure 3, Step 7).

k. Discard the reconstitution collar and glass vial (Figure 3, Step 8).

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.*

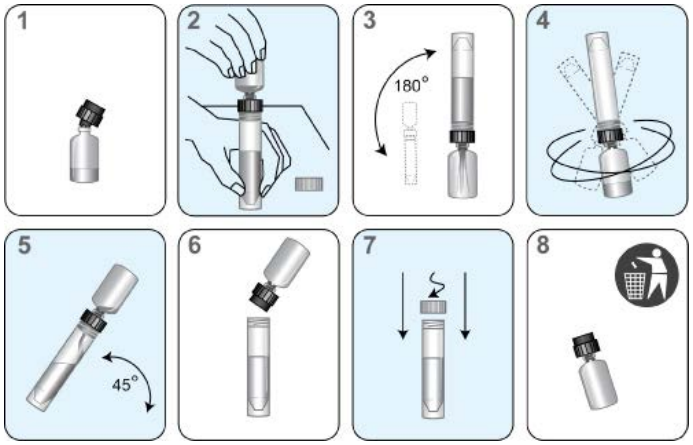

Figure 3. Panther System Reconstitution Process

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

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

D. Specimen Handling

1. Allow the controls and specimens to reach room temperature prior to processing.
2. Do not vortex specimens.
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single pink Aptima collection swab in a multitest or vaginal swab specimen transport tube.
   b. A final volume of urine between the black fill lines of a urine specimen transport tube.
   c. The presence of a single blue Aptima collection swab in a unisex swab specimen transport tube.
   d. The absence of a swab in the Aptima specimen transport tube for PreservCyt solution liquid Pap specimens.
4. Inspect specimen tubes before loading into 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 4a–c may result in liquid discharge from the specimen tube cap.*

Note: *Up to 4 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 4 aliquots from the specimen tube can lead to processing errors.*

E. System Preparation

1. Set up the system according to the instructions in the *Panther System Operator's Manual* and *Procedural Notes*. 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 for 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. Control results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

B. Temperature

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

C. Glove Powder

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

D. Lab Contamination Monitoring Protocol for the Panther System

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

To monitor for laboratory contamination, the following procedure may be performed using the Aptima unisex awab specimen collection kit for endocervical and male urethral swab specimens.

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the 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.

If the results are CT positive or equivocal, 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 Aptima Assay software using the CT protocol. 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.

Test Interpretation	Total RLU (x1000)
Negative	0* to < 50
Equivocal	50 to < 100
Low RLU Positive[1, 2, 3]	100 to < 5,000
Positive[1, 2]	5,000 to < 12,000
Invalid	0* or > 12,000

* A zero (0 x 1000) RLU result on the run report represents a value between zero and 999 RLU. RLU values less than 160 on DTS systems or 690 on the Tigris DTS system or Panther system will be reported as invalid.

[1] According to CDC guidelines, "consideration should be given to routine additional testing for persons with positive CT or GC screening tests when risk-factor information or actual surveys indicate that the prevalence is low, resulting in a lower PPV (e.g., < 90%)." Refer to CDC guidelines for details on additional testing and patient management after a positive screening test (4).

[2] Refer to Table 3 for RLU distribution of results. The magnitude of RLU is not indicative of the level of organism in the specimen.

[3] 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 GC 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, a final volume of urine in between the black fill lines of a urine specimen transport tube, or the absence of a swab in an Aptima specimen transfer tube for liquid Pap specimens.

The Positive 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

* A zero (0 x 1000) RLU result on the run report represents a value between zero and 999 RLU. RLU values less than 160 on DTS systems or 690 on the Tigris DTS system or Panther system will be reported as invalid.

1. The Aptima assay software automatically evaluates the controls according to the above criteria and the results will be reflected in the results report.
2. If the Run Status is FAIL, all test results in the same run are invalid and must not be reported.
3. Each laboratory should implement appropriate control procedures to satisfy the requirements of CLIA regulations.

Note: *See Troubleshooting, or contact Hologic Technical Support for help with out-of-range controls on the DTS systems.*

4. A Tigris DTS system parameter permits each site to specify a "control bracketing" frequency whereby additional sets of controls can be placed at defined intervals within the worklist. If this parameter is specified, the Tigris DTS system will require a set of controls to be placed after the defined number of specimens in the control bracket. The Tigris DTS system automatically evaluates each control in the worklist according to the above criteria and will invalidate all specimens in the affected control bracket(s) if the control criteria are not met. See the *Tigris DTS System Operator's Manual* for additional details.
5. Negative controls may not be effective in monitoring random carryover. See *Tigris DTS System Analytical Performance* for results from a high-target analytical carryover study that was performed to demonstrate control of carryover on the Tigris DTS system. 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, urine, and PreservCyt solution liquid Pap 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.
- Testing of an endocervical specimen is recommended for female patients who are clinically suspected of having a chlamydial or gonococcal infection. If both a Pap and endocervical swab are collected, the PreservCyt solution liquid Pap specimen must be collected before the endocervical swab specimen.

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. The presence of mucus in endocervical specimens does not interfere with the detection of CT by the Aptima CT assay. However, to ensure collection of cells infected with CT, columnar epithelial cells lining the endocervix should be sampled. If excess mucus is not removed, sampling of these cells is not ensured.

D. Urine, vaginal swab, and PreservCyt solution liquid Pap specimen sampling is not designed to replace cervical exams and endocervical specimens for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.

E. The Aptima CT assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications. For those patients for whom a false positive result may have adverse psycho-social impact, CDC recommends retesting by a method using an alternate technology (4).

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

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

H. Results from the Aptima CT assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

I. A negative result does not preclude a possible infection because results are dependent on adequate specimen collection. Test results may be affected by improper specimen collection, technical error, specimen mix-up, or target levels below the assay limit of detection.

J. 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.

K. For the vaginal swab, endocervical swab, male urethral swab and urine specimen clinical studies, 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.

L. For the PreservCyt solution liquid Pap specimen clinical studies, the Aptima CT assay performance for detecting CT is derived primarily from low prevalence populations. Nonetheless, 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.

M. Performance of the Aptima specimen transfer kit was not evaluated for testing the same PreservCyt solution liquid Pap specimen both before and after ThinPrep Pap processing.

N. PreservCyt solution liquid Pap specimens processed with instruments other than the ThinPrep 2000 processor have not been evaluated for use in Aptima assays.

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

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

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

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

S. The performance of endocervical, vaginal, and male urethral swab specimens, male and female urine specimens, and PreservCyt solution liquid Pap specimens has not been evaluated in adolescents less than 16 years of age.

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

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

V. There is no evidence of degradation of nucleic acids in PreservCyt solution. If a PreservCyt solution liquid Pap specimen has small numbers of CT cellular material, uneven distribution of this cellular material may occur. Also, when compared to direct sampling with the Aptima STM, the additional volume of PreservCyt solution results in greater dilution of the sample material. These factors may affect the ability to detect small numbers of organisms in the collected material. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary.

W. Customers must independently validate an LIS transfer process.

Clinical Study Results

The performance of the Aptima CT assay was established in two multi-center clinical investigations conducted in North America. In the first clinical investigation, two studies were conducted. First, the clinical specimen study established the sensitivity, specificity, and predictive values of the Aptima CT assay using clinician-collected endocervical, vaginal, and male urethral swab specimens, patient-collected vaginal swab specimens, and male and female urine specimens. The second study, in the first clinical investigation, evaluated the precision of the Aptima CT assay when performed according to NCCLS Guidelines (17). The second clinical investigation established the sensitivity, specificity, and predictive values of the Aptima CT assay using PreservCyt solution (component of the ThinPrep™ 2000 System). PreservCyt solution liquid Pap specimens were also evaluated for within-laboratory precision with the Aptima CT assay.

DTS Systems Expected Values

Prevalence

The prevalence of CT in patient populations depends on risk factors such as age, gender, the presence of symptoms, the type of clinic, and the test method. A summary of the prevalence of CT, by specimen type as determined by the Aptima CT assay is shown in Tables 1a and 1b for two multi-center clinical investigations by clinical site and overall.

Table 1a: Prevalence of C. trachomatis by Clinical Site and Overall as Determined by Aptima CT Assay Results

Site	% (#positive / #tested)											
	MS		MU		FS		FU		PVS		CVS	
1	27.0	(68/252)	25.0	(63/252)	16.5	(38/230)	17.0	(39/229)	19.2	(42/219)	19.1	(44/230)
2	27.7	(98/354)	26.6	(94/354)	35.0	(70/200)	26.5	(53/200)	30.8	(61/198)	33.0	(66/200)
3	25.0	(1/4)	25.0	(1/4)	11.4	(13/114)	8.8	(10/113)	10.8	(12/111)	11.5	(13/113)
4	N/A	N/A	N/A	N/A	11.6	(31/267)	8.1	(22/271)	9.3	(25/268)	12.2	(33/270)
5	8.0	(16/200)	8.0	(16/200)	9.0	(18/199)	7.5	(15/199)	8.0	(16/199)	10.1	(20/199)
6	22.7	(69/304)	20.0	(61/305)	14.3	(42/294)	13.2	(39/295)	15.2	(44/290)	16.2	(48/296)
7	5.8	(12/207)	6.3	(13/207)	7.8	(8/102)	9.8	(10/102)	12.7	(13/102)	8.8	(9/102)
8	N/A	N/A	N/A	N/A	8.2	(4/49)	6.1	(3/49)	12.5	(6/48)	7.8	(4/51)
All	20.0	(264/1321)	18.8	(248/1322)	15.4	(224/1455)	13.1	(191/1458)	15.3	(219/1435)	16.2	(237/1461)

MS = male urethral swab; **MU** = male urine; **FS** = female endocervical swab; **FU** = female urine; **PVS** = patient-collected vaginal swab; **CVS** = slinician-collected aginal swab.

Table 1b: Prevalence of C. trachomatis by Clinical Site and Overall as Determined by Aptima CT Assay Results Using PreservCyt Solution Liquid Pap Specimens

Site	% (#positive / #tested)	
1	17.0	(17/100)
2	3.2	(4/124)
3	7.4	(35/475)
4	4.2	(12/287)
5	5.4	(16/297)
6	5.5	(20/364)
All	6.3	(104/1647)

Positive and Negative Predictive Values for Hypothetical Prevalence Rates in North America

The estimated positive and negative predictive values (PPV and NPV) for different hypothetical prevalence rates using the Aptima CT assay are shown in Table 2. These calculations are based on hypothetical prevalence rates and the overall sensitivity and specificity estimated from the patient infected status for three multi-center clinical investigations. The overall sensitivity and specificity for CT were 96.7% and 96.8%,

respectively (Table 2). The actual PPV and NPV for clinician-collected endocervical, vaginal and male urethral swab, patient-collected vaginal swab, and male and female urine specimens are shown in Table 6 for each clinical site and overall. The actual PPV and NPV for PreservCyt solution liquid Pap specimens are shown in Table 6a.

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

Hypothetical Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	96.7	96.8	23.5	100.0
2	96.7	96.8	38.3	99.9
5	96.7	96.8	61.6	99.8
10	96.7	96.8	77.2	99.6
15	96.7	96.8	84.3	99.4
20	96.7	96.8	88.4	99.2
25	96.7	96.8	91.0	98.9
30	96.7	96.8	92.9	98.6

Aptima CT Assay RLU Distribution

Figure 4 shows the RLU distribution for the Aptima CT assay for all specimen types in the clinical study except PreservCyt solution liquid Pap specimens. Table 3 summarizes the RLU distribution for the total positive and total negative results, as well as the false positive and false negative results for each specimen type except PreservCyt solution liquid Pap specimens relative to infected patient status. Across certain specimen types, there is a trend toward an increasing proportion of true positives as the RLU values increase.

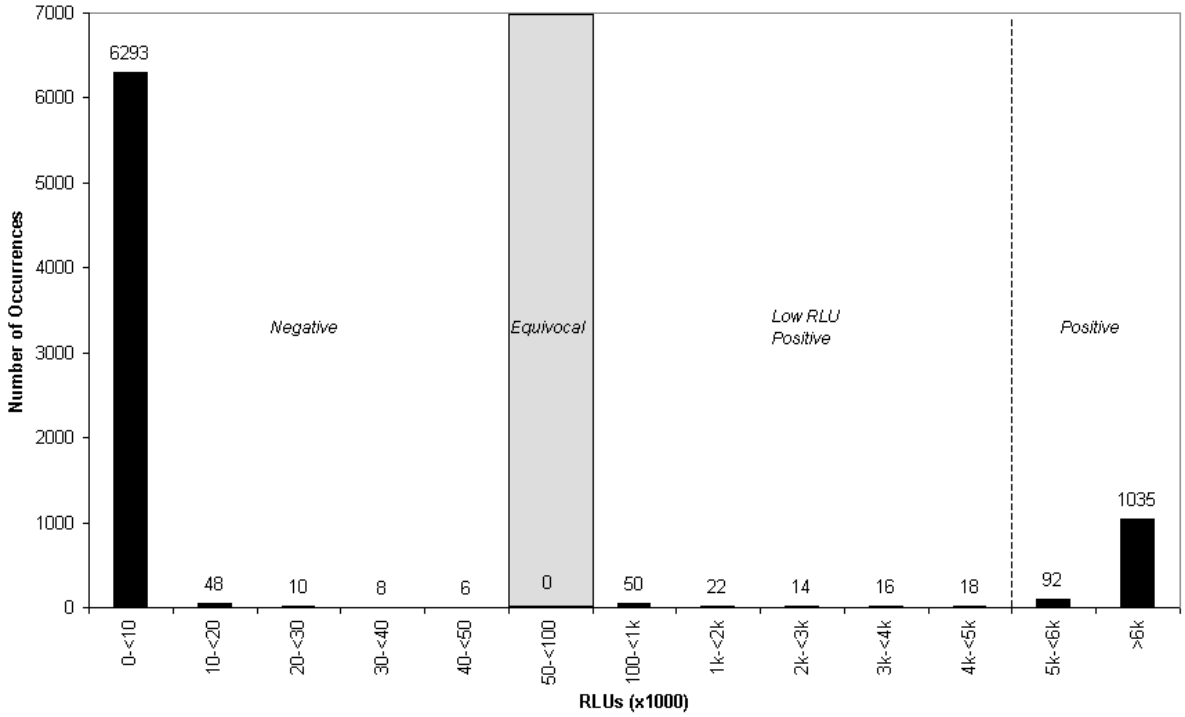

Figure 4. Frequency of RLU Distribution for the Aptima CT Assay

Table 3: Aptima CT Assay RLU Distribution

	RLUs (x 1000)												
	0 < 10	**10 < 20**	**20 < 30**	**30 < 40**	**40 < 50**	**50 < 100**	**100 < 1000**	**1000 < 2000**	**2000 < 3000**	**3000< 4000**	**4000 < 5000**	**5000 < 6000**	**> 6000**
Total Positives						0	**50**	**22**	**14**	**16**	**18**	**92**	**1035**
Total False Positives						0	**43**	**17**	**7**	**11**	**10**	**25**	**126**
CVS						0	18	4	1	4	4	6	28
PVS						0	7	5	2	1	2	2	6
FS						0	9	2	3	2	2	5	26
MS						0	3	4	0	1	0	3	32
FU						0	5	2	0	1	0	6	12
MU						0	1	0	1	2	2	3	22
Total Negatives	**6293**	**48**	**10**	**8**	**6**	0							
Total False Negatives	**31**	**1**	**0**	**1**	**0**	0							
CVS	4	0	0	1	0	0							
PVS	1	0	0	0	0	0							
FS	3	0	0	0	0	0							
MS	4	1	0	0	0	0							
FU	10	0	0	0	0	0							
MU	9	0	0	0	0	0							

CVS = clinician-collected vaginal swab; **PVS** = asymptomatic patient-collected vaginal swab; **FS** = female endocervical swab; **MS** = male urethral swab; **FU** = female urine; **MU** = male urine; **RLU** = relative light unit.

Shaded column denotes equivocal zone.

DTS Systems Clinical Performance

See *Tigris DTS System Clinical Specimen Agreement* following the *DTS Systems Analytical Performance* section for Tigris DTS system-specific clinical performance.

Clinical Specimen Study — Endocervical Swab, Male Urethral Swab, Vaginal Swab, and Urine Specimens

Clinician-collected endocervical, vaginal and male urethral swab, patient-collected vaginal swab, and male and female urine specimens were collected from 2,787 symptomatic and asymptomatic, male and female subjects attending OB/GYN, sexually transmitted disease (STD), teen, and family planning clinics at eight geographically diverse clinical sites in North America. Subjects were classified as symptomatic if symptoms such as discharge, dysuria, and pelvic pain were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. Of the 1,392 asymptomatic subjects enrolled in the study, 2 were less than 16 years of age, 237 were between the ages of 16 and 20, 423 were between the ages of 21 and 25, and 730 were greater than 25 years of age. Of the 1,395 symptomatic subjects enrolled in the study, 211 were between the ages of 16 and 20, 494 were between the ages of 21 and 25, and 690 were greater than 25 years of age.

Three specimens were collected from each of the 1,322 eligible male subjects. Five specimens were collected from each of the 1,465 eligible female subjects. For male subjects, two randomized urethral swabs were collected followed by one urine specimen. For female subjects, one urine specimen was collected followed by one patient-collected vaginal swab, one clinician-collected vaginal swab, and two randomized endocervical swabs. Aptima CT assay and Aptima Combo 2 assay CT results were generated from the two vaginal swabs, one endocervical swab, one male urethral swab, and a male and female urine aliquot. The remaining endocervical swab, male urethral swab, and a male and female urine aliquot were tested using another commercially-available NAAT. Endocervical and male urethral swab specimens and male and female urine specimens tested in the Aptima Combo 2 assay and the other commercially available NAAT were used as the reference NAATs to determine infected status for each subject. Specimen testing was conducted either at the site of subject enrollment or at an external testing site.

All performance calculations were based on the total number of Aptima CT assay results for endocervical, vaginal and male urethral swab, and male and female urine specimens compared to a patient infected status algorithm for each gender. In the algorithm, the designation of a subject as being infected or not infected with CT was based on endocervical swab and urine specimen results from the commercially-available Aptima Combo 2 assay and the other commercially-available NAAT. Subjects were considered infected with CT if two of the four endocervical swab and urine specimens tested positive in the Aptima Combo 2 assay and the other reference NAAT (one specimen testing positive in each NAAT). Subjects were considered non-infected if less than two reference NAAT results were positive.

A total of 8,406 Aptima CT assay results were used to calculate sensitivity and specificity. Sensitivity and specificity for CT by gender, specimen type and symptom status are presented in Table 4. Table 6 shows the Aptima CT assay sensitivity, specificity, and predictive values compared to patient infected status for each clinical site and overall. Tables 7a–7d summarize the number of results from symptomatic and asymptomatic subjects designated as infected or non-infected with CT according to the patient infected status algorithm.

Of the 2,787 subjects enrolled, there were 13 subjects with unknown CT patient infected status. Subjects were designated with an unknown patient infected status if results were missing that prevented conclusive determination of infected status. These subjects' results were not included in any performance calculations. Of the 8,452 Aptima CT assay results from the multi-center clinical study, there was a small percentage (8, 0.09%) of specimens that initially tested invalid for CT. Upon repeat testing, there were no equivocal or invalid results.

Table 4: Sensitivity and Specificity of the Aptima CT Assay Relative to Patient Infected Status by Symptom Status and Overall

Specimen		Symptom Status	N	TP	FP	TN	FN	Sensitivity (95% CI)		Specificity (95% CI)	
Male	Swab	Symptomatic	576	131	23[a]	418	4	97.0	(92.6–99.2)	94.8	(92.3–96.7)
		Asymptomatic	745	90	20[b]	634	1	98.9	(94.0–100)	96.9	(95.3–98.1)
		All	1321	221	43[c]	1052	5	97.8	(94.9–99.3)	96.1	(94.7–97.1)
	Urine	Symptomatic	576	127	14[d]	427	8	94.1	(88.7–97.4)	96.8	(94.7–98.3)
		Asymptomatic	746	90	17[e]	638	1	98.9	(94.0–100)	97.4	(95.9–98.5)
		All	1322	217	31[f]	1065	9	96.0	(92.6–98.2)	97.2	(96.0–98.1)
Female	Swab	Symptomatic	807	114	28[g]	664	1	99.1	(95.3–100)	96.0	(94.2–97.3)
		Asymptomatic	636	59	22[h]	553	2	96.7	(88.7–99.6)	96.2	(94.3–97.6)
		All	1443	173	50[i]	1217	3	98.3	(95.1–99.6)	96.1	(94.8–97.1)
	Urine	Symptomatic	809	107	13[j]	682	7	93.9	(87.8–97.5)	98.1	(96.8–99.0)
		Asymptomatic	639	58	13[k]	565	3	95.1	(86.3–99.0)	97.8	(96.2–98.8)
		All	1448	165	26[l]	1247	10	94.3	(89.7–97.2)	98.0	(97.0–98.7)
Patient-Collected	Vaginal Swab	Asymptomatic	629	60	25[m]	543	1	98.4	(91.2–100)	95.6	(93.6–97.1)
Clinician-Collected	Vaginal Swab	Symptomatic	811	111	33[n]	663	4	96.5	(91.3–99.0)	95.3	(93.4–96.7)
		Asymptomatic	638	60	32[o]	545	1	98.4	(91.2–99.0)	94.5	(92.3–96.2)
		All	1449	171	65[p]	1208	5	97.2	(93.5–99.1)	94.9	(93.5–96.0)

TP = true positive; **FP** = false positive; **TN** = true negative; **FN** = false negative.

Aptima Combo 2 assay CT results: # positive results / # specimens tested [a]9/23; [b]14/20; [c]23/43; [d]6/14; [e]6/17; [f]12/31; [g]14/28; [h]11/22; [i]25/50; [j]7/13; [k]5/13; [l]12/26; [m]15/25; [n]17/33; [o]15/32; [p]32/65.

Clinical Specimen Study — PreservCyt Solution Liquid Pap

A prospective multi-center clinical study was conducted to evaluate the use of the PreservCyt Solution (a component of the ThinPrep 2000 system) as an alternative medium for gynecological specimens for the detection of CT by the Aptima CT assay. One thousand six hundred forty-seven (1,647) symptomatic and asymptomatic female subjects attending OB/GYN, family planning, public health, women's, and STD clinics were evaluated in the clinical study. Of the 1,647 evaluable subjects, 1,288 were asymptomatic subjects and 359 were symptomatic subjects. Subjects were enrolled from sites with CT prevalence that ranged from 2.8% to 14.0%.

Two specimens were collected from each eligible subject: one PreservCyt solution liquid Pap specimen and one endocervical swab specimen. PreservCyt solution liquid Pap specimens were collected with the spatula/cyto-brush or a broom-like brush cervical sampling device. The distribution of cervical sampling devices is summarized in Table 5 by specimen collection site and overall.

PreservCyt solution liquid Pap specimens were processed in accordance with the ThinPrep 2000 Processor Operator's Manual and Aptima Specimen Transfer Kit Package Insert. After processing the PreservCyt solution liquid Pap specimen with the ThinPrep 2000 Processor, the specimen was transferred into the Aptima specimen transfer kit for testing with the Aptima CT assay.

Sensitivity and specificity of the Aptima CT assay in PreservCyt solution liquid Pap specimens were calculated by comparing results to a patient infected status algorithm. The algorithm included Aptima Combo 2 assay and Aptima CT assay results in endocervical swab specimens. Both reference NAATs were required to be positive to establish an infected patient status. At least one reference NAAT was required to be negative to establish a non-infected patient status. Table 7e summarizes the frequency of test outcomes for the two reference NAATs.

Table 5a shows the sensitivities and specificities of the Aptima CT assay by symptom status and overall. Overall sensitivity was 95.6% (86/90). In symptomatic and asymptomatic subjects, sensitivities were 96.7% (29/30) and 95.0% (57/60), respectively. Overall specificity was 98.8% (1539/1557). In symptomatic and asymptomatic subjects, specificities were 98.8% (325/329) and 98.9% (1214/1228), respectively.

Table 6a shows the sensitivities and specificities of the Aptima CT assay by specimen collection site and overall. Sensitivities ranged from 92.9% to 100%. Specificities ranged from 96.5% to 100%.

Table 5: Distribution of Cervical Sampling Device Used for PreservCyt Solution Liquid Pap Specimens

Cervical Sampling Device Used	Clinical Collection Site						Total
	1	2	3	4	5	6	
Spatula/Cytobrush	0	124	475	287	57	364	1307
Broom-Type Device	100	0	0	0	240	0	340

Table 5a: Sensitivity and Specificity of the Aptima CT Assay Relative to Patient Infected Status by Symptom Status and Overall for PreservCyt Solution Liquid Pap Specimens

Specimen	Aptima CT PreservCyt Solution Result	+/+	+/-	-/+	-/-	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Symptomatic	Positive	29	0	1	3	96.7 (29/30) (82.8–99.9)	98.8 (325/329) (96.9–99.7)
	Negative	1	3	3	319		
	Total	30	3	4	322		
Asymptomatic	Positive	57	0	1	13	95.0 (57/60) (86.1–99.0)	98.9 (1214/1228) (98.1–99.4)
	Negative	3	2	11	1201		
	Total	60	2	12	1214		
All	Positive	86	0	2	16	95.6 (86/90) (89.0–98.8)	98.8 (1539/1557) (98.2–99.3)
	Negative	4	5	14	1520		
	Total	90	5	16	1536		

CI = confidence interval.
+/+ = Positive endocervical swab specimen result in the Aptima Combo 2 Assay/Positive endocervical swab specimen result in the Aptima CT Assay.
+/- = Positive endocervical swab specimen result in the Aptima Combo 2 Assay/Negative endocervical swab specimen result in the Aptima CT Assay.
-/+ = Negative endocervical swab specimen result in the Aptima Combo 2 Assay/Positive endocervical swab specimen result in the Aptima CT Assay.
-/- = Negative endocervical swab specimen result in the Aptima Combo 2 Assay/Negative endocervical swab specimen result in the Aptima CT Assay.

Table 6: Sensitivity, Specificity and Predictive Values of the Aptima CT Assay Relative to Patient Infected Status by Clinical Site and Overall

Specimen		Site	N	TP	FP	TN	FN	Prev. (%)	Sensitivity (95% CI)		Specificity (95% CI)		PPV (%)	NPV (%)
Male	Swab	1	252	54	14	183	1	21.8	98.2	(90.3–100)	92.9	(88.4–96.1)	79.4	99.5
		2	354	83	15	252	4	24.6	95.4	(88.6–98.7)	94.4	(90.9–96.8)	84.7	98.4
		3	4	1	0	3	0	25.0	100	(2.5–100)	100	(29.2–100)	100	100
		4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		5	200	12	4	184	0	6.0	100	(73.5–100)	97.9	(94.6–99.4)	75.0	100
		6	304	59	10	235	0	19.4	100	(93.9–100)	95.9	(92.6–98.0)	85.5	100
		7	207	12	0	195	0	5.8	100	(73.5–100)	100	(98.1–100)	100	100
		8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		All	1321	221	43	1052	5	17.1	97.8	(94.9–99.3)	96.1	(94.7–97.1)	83.7	99.4
	Urine	1	252	54	9	188	1	21.8	98.2	(90.3–100)	95.4	(91.5–97.9)	85.7	99.5
		2	354	85	9	258	2	24.6	97.7	(91.9–99.7)	96.6	(93.7–98.4)	90.4	99.2
		3	4	1	0	3	0	25.0	100	(2.5–100)	100	(29.2–100)	100	100
		4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		5	200	12	4	184	0	6.0	100	(73.5–100)	97.9	(94.6–99.4)	75.0	100
		6	305	53	8	238	6	19.3	89.8	(79.2–96.2)	96.7	(93.7–98.6)	86.9	97.5
		7	207	12	1	194	0	5.8	100	(73.5–100)	99.5	(97.2–100)	92.3	100
		8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		All	1322	217	31	1065	9	17.1	96.0	(92.6–98.2)	97.2	(96.0–98.1)	87.5	99.2
Female	Swab	1	228	36	2	190	0	15.8	100	(90.3–100)	99.0	(96.3–99.9)	94.7	100
		2	198	52	18	128	0	26.3	100	(93.2–100)	87.7	(81.2–92.5)	74.3	100
		3	114	9	4	101	0	7.9	100	(66.4–100)	96.2	(90.5–99.0)	69.2	100
		4	260	19	11	229	1	7.7	95.0	(75.1–99.9)	95.4	(91.9–97.7)	63.3	99.6
		5	199	13	5	181	0	6.5	100	(75.3–100)	97.3	(93.8–99.1)	72.2	100
		6	294	33	9	252	0	11.2	100	(89.4–100)	96.6	(93.6–98.4)	78.6	100
		7	102	8	0	92	2	9.8	80.0	(44.4–97.5)	100	(96.1–100)	100	97.9
		8	48	3	1	44	0	6.3	100	(29.2–100)	97.8	(88.2–99.9)	75.0	100
		All	1443	173	50	1217	3	12.2	98.3	(95.1–99.6)	96.1	(94.8–97.1)	77.6	99.8
	Urine	1	227	34	5	187	1	15.4	97.1	(85.1–99.9)	97.4	(94.0–99.1)	87.2	99.5
		2	198	51	2	144	1	26.3	98.1	(89.7–100)	98.6	(95.1–99.8)	96.2	99.3
		3	113	9	1	103	0	8.0	100	(66.4–100)	99.0	(94.8–100)	90.0	100
		4	265	18	4	241	2	7.5	90.0	(68.3–98.8)	98.4	(95.9–99.6)	81.8	99.2
		5	199	11	4	182	2	6.5	84.6	(54.6–98.1)	97.8	(94.6–99.4)	73.3	98.9
		6	295	29	10	252	4	11.2	87.9	(71.8–96.6)	96.2	(93.1–98.2)	74.4	98.4
		7	102	10	0	92	0	9.8	100	(69.2–100)	100	(96.1–100)	100	100
		8	49	3	0	46	0	6.1	100	(29.2–100)	100	(92.3–100)	100	100
		All	1448	165	26	1247	10	12.1	94.3	(89.7–97.2)	98.0	(97.0–98.7)	86.4	99.2

Table 6: Sensitivity, Specificity and Predictive Values of the Aptima CT Assay Relative to Patient Infected Status by Clinical Site and Overall (continued)

Specimen		Site	N	TP	FP	TN	FN	Prev. (%)	Sensitivity (95% CI)		Specificity (95% CI)		PPV (%)	NPV (%)
Patient-Collected	Vaginal Swab	1	70	14	4	52	0	20.0	100	(76.8–100)	92.9	(82.7–98.0)	77.8	100
		2	46	13	4	29	0	28.3	100	(75.3–100)	87.9	(71.8–96.6)	76.5	100
		3	45	4	2	39	0	8.9	100	(39.8–100)	95.1	(83.5–99.4)	66.7	100
		4	152	6	3	142	1	4.6	85.7	(42.1–99.6)	97.9	(94.1–99.6)	66.7	99.3
		5	130	7	3	120	0	5.4	100	(59.0–100)	97.6	(93.0–99.5)	70.0	100
		6	75	8	5	62	0	10.7	100	(63.1–100)	92.5	(83.4–97.5)	61.5	100
		7	68	5	2	61	0	7.4	100	(47.8–100)	96.8	(89.0–99.6)	71.4	100
		8	43	3	2	38	0	7.0	100	(29.2–100)	95.0	(83.1–99.4)	60.0	100
		All	629	60	25	543	1	9.7	98.4	(91.2–100)	95.6	(93.6–97.1)	70.6	99.8
Clinician-Collected	Vaginal Swab	1	228	36	8	184	0	15.8	100	(90.3–100)	95.8	(92.0–98.2)	81.8	100
		2	198	50	16	130	2	26.3	96.2	(86.8–99.5)	89.0	(82.8–93.6)	75.8	98.5
		3	113	9	4	100	0	8.0	100	(66.4–100)	96.2	(90.4–98.9)	69.2	100
		4	263	18	14	229	2	7.6	90.0	(68.3–98.8)	94.2	(90.5–96.8)	56.3	99.1
		5	199	13	7	179	0	6.5	100	(75.3–100)	96.2	(92.4–98.5)	65.0	100
		6	296	33	15	248	0	11.1	100	(89.4–100)	94.3	(90.8–96.8)	68.8	100
		7	102	9	0	92	1	9.8	90.0	(55.5–99.7)	100	(96.1–100)	100	98.9
		8	50	3	1	46	0	6.0	100	(29.2–100)	97.9	(88.7–99.9)	75.0	100
		All	1449	171	65	1208	5	12.1	97.2	(93.5–99.1)	94.9	(93.5–96.0)	72.5	99.6

TP = true positive; **FP** = false positive; **TN** = true negative; **FN** = false negative; **Prev** = prevalence**; CI** = confidence interval; **PPV** = positive predictive value; **NPV** = negative predictive value.

Table 6a: Sensitivity, Specificity and Predictive Values of the Aptima CT Assay Relative to Patient Infected Status by Clinical Site and Overall for PreservCyt Solution Liquid Pap Specimens

Site	Aptima CT PreservCyt Solution Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)
1	Positive	14	0	1	2	14.0	100 (14/14) (76.8–100)	96.5 (83/86) (90.1–99.3)	82.4	100
	Negative	0	0	0	83					
	Total	14	0	1	85					
2	Positive	4	0	0	0	3.2	100 (4/4) (39.8–100)	100 (120/120) (97.0–100)	100	100
	Negative	0	0	2	118					
	Total	4	0	2	118					
3	Positive	29	0	0	6	6.5	93.5 (29/31) (78.6–99.2)	98.6 (438/444) (97.1–99.5)	82.9	99.5
	Negative	2	0	2	436					
	Total	31	0	2	442					
4	Positive	8	0	0	4	2.8	100 (8/8) (63.1–100)	98.6 (275/279) (96.4–99.6)	66.7	100
	Negative	0	3	1	271					
	Total	8	3	1	275					
5	Positive	13	0	0	3	4.7	92.9 (13/14) (66.1–99.8)	98.9 (280/283) (96.9–99.8)	81.3	99.6
	Negative	1	1	4	275					
	Total	14	1	4	278					
6	Positive	18	0	1	1	5.2	94.7 (18/19) (74.0–99.9)	99.4 (343/345) (97.9–99.9)	90.0	99.7
	Negative	1	1	5	337					
	Total	19	1	6	338					
All	Positive	86	0	2	16	5.5	95.6 (86/90) (89.0–98.8)	98.8 (1539/1557) (98.2–99.3)	82.7	99.7
	Negative	4	5	14	1520					
	Total	90	5	16	1536					

Prev = prevalence; **CI** = confidence interval; **PPV** = positive predictive value; **NPV** = negative predictive value.

+/+ = Positive endocervical swab specimen result in the Aptima Combo 2 Assay/Positive endocervical swab specimen result in the Aptima CT Assay.

+/- = Positive endocervical swab specimen result in the Aptima Combo 2 Assay/Negative endocervical swab specimen result in the Aptima CT Assay.

-/+ = Negative endocervical swab specimen result in the Aptima Combo 2 Assay/Positive endocervical swab specimen result in the Aptima CT Assay.

-/- = Negative endocervical swab specimen result in the Aptima Combo 2 Assay/Negative endocervical swab specimen result in the Aptima CT Assay.

Table 7a: Male Urethral Swab and Urine Results from Subjects Infected or Non-Infected with C. trachomatis According to Patient Infected Status

Patient Infected Status	NAAT 1 (Aptima Combo 2 Assay)		NAAT 2		Aptima CT Assay		Symptom Status		Total
	MS	MU	MS	MU	MS	MU	Sym	Asym	
Infected	+	+	+	+	+	+	96	68	164
Infected	+	+	+	+	+	-	5	1	6
Infected	+	+	+	-	+	+	11	7	18
Infected	+	+	-	+	+	+	13	11	24
Infected	+	+	-	+	+	-	1	0	1
Infected	+	+	-	+	-	+	1	0	1
Infected	+	-	+	+	+	+	2	0	2
Infected	+	-	+	+	+	-	1	0	1
Infected	+	-	+	-	+	-	1	0	1
Infected	-	+	+	+	+	+	1	0	1
Infected	-	+	-	+	+	+	0	2	2
Infected	-	+	-	+	-	+	3	1	4
Infected	-	+	=	+	+	+	0	1	1
Non-infected	+	+	-	-	+	+	4	4	8
Non-infected	+	+	-	-	-	+	1	0	1
Non-infected	+	-	-	-	+	+	1	4	5
Non-infected	+	-	-	-	+	-	4	6	10
Non-infected	+	-	-	-	-	+	1	0	1
Non-infected	+	-	-	-	-	-	3	0	3
Non-infected	-	+	-	-	+	+	1	0	1
Non-infected	-	+	-	-	-	+	0	2	2
Non-infected	-	+	-	-	-	-	1	0	1
Non-infected	-	-	+	+	+	+	1	0	1
Non-infected	-	-	-	+	-	-	2	2	4
Non-infected	-	-	-	-	+	+	1	1	2
Non-infected	-	-	-	-	+	-	11	5	16
Non-infected	-	-	-	-	-	+	4	4	8
Non-infected	-	-	-	-	-	-	403	618	1021
Non-infected	-	-	-	N/A	-	+	0	2	2
Non-infected	-	-	-	N/A	-	-	1	2	3
Non-infected	-	-	-	=	-	-	0	4	4
Non-infected	-	-	=	-	-	-	2	0	2
Non-infected	N/A	-	-	-	N/A	-	0	1	1
Total							576	746	1322

N/A = specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.

MS = male urethral swab; **MU** = male urine; **Sym** = symptomatic; **Asym** = asymptomatic.

Table 7b: Female Endocervical Swab and Urine Results from Subjects Infected or Non-Infected with C. trachomatis According to Patient Infected Status

Patient Infected Status	NAAT 1 (Aptima Combo 2 Assay)		NAAT 2		Aptima CT Assay		Symptom Status		Total
	FS	FU	FS	FU	FS	FU	Sym	Asym	
Infected	+	+	+	+	+	+	80	43	123
Infected	+	+	+	+	+	-	1	1	2
Infected	+	+	+	-	+	+	10	5	15
Infected	+	+	+	=	+	+	1	0	1
Infected	+	+	-	+	+	+	9	3	12
Infected	+	-	+	+	+	+	3	1	4
Infected	+	-	+	+	+	-	2	2	4
Infected	+	-	+	-	+	+	2	0	2
Infected	+	-	+	-	+	-	4	0	4
Infected	+	-	+	-	+	N/A	1	0	1
Infected	-	+	+	+	+	+	0	1	1
Infected	-	+	-	+	+	+	1	3	4
Infected	-	+	-	+	-	+	1	2	3
Non-infected	+	+	-	-	+	+	1	2	3
Non-infected	+	+	-	N/A	+	+	1	0	1
Non-infected	+	-	-	-	+	+	0	2	2
Non-infected	+	-	-	-	+	-	12	7	19
Non-infected	+	-	-	-	-	-	0	1	1
Non-infected	-	+	-	-	+	+	1	0	1
Non-infected	-	+	-	-	-	+	4	3	7
Non-infected	-	+	-	-	-	-	0	1	1
Non-infected	-	-	+	-	-	-	1	1	2
Non-infected	-	-	-	+	-	-	1	2	3
Non-infected	-	-	-	-	+	+	0	2	2
Non-infected	-	-	-	-	+	-	11	9	20
Non-infected	-	-	-	-	-	+	5	4	9
Non-infected	-	-	-	-	-	-	636	526	1162
Non-infected	-	-	-	-	-	N/A	1	0	1
Non-infected	-	-	-	N/A	-	-	2	3	5
Non-infected	-	-	-	=	-	-	12	10	22
Non-infected	-	-	=	-	-	-	1	1	2
Non-infected	-	N/A	-	-	-	N/A	1	1	2
Non-infected	N/A	-	-	-	N/A	-	5	4	9
Non-infected	=	-	-	-	+	+	1	0	1
Non-infected	=	-	-	-	+	-	1	0	1
Total							812	640	1452

N/A = specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.

FS = female endocervical swab; **FU** = female urine; **Sym** = symptomatic; **Asym** = asymptomatic.

Table 7c: Asymptomatic Patient-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with C. trachomatis According to Patient Infected Status

Patient Infected Status	NAAT 1 (Aptima Combo 2 Assay)		NAAT 2		Aptima CT Assay	Total
	FS	FU	FS	FU	PVS	
Infected	+	+	+	+	+	44
Infected	+	+	+	-	+	5
Infected	+	+	-	+	+	3
Infected	+	-	+	+	+	3
Infected	-	+	+	+	+	1
Infected	-	+	-	+	+	4
Infected	-	+	-	+	-	1
Non-infected	+	+	-	-	+	2
Non-infected	+	-	-	-	+	4
Non-infected	+	-	-	-	+	1
Non-infected	+	-	-	-	-	2
Non-infected	+	-	-	-	-	3
Non-infected	-	+	-	-	+	2
Non-infected	-	+	-	-	-	2
Non-infected	-	-	+	-	-	1
Non-infected	-	-	-	+	-	2
Non-infected	-	-	-	-	+	5
Non-infected	-	-	-	-	+	10
Non-infected	-	-	-	-	-	15
Non-infected	-	-	-	-	-	500
Non-infected	-	-	-	-	-	1
Non-infected	-	-	-	-	N/A	1
Non-infected	-	-	-	-	N/A	9
Non-infected	-	-	-	N/A	-	2
Non-infected	-	-	-	N/A	N/A	1
Non-infected	-	-	-	=	-	1
Non-infected	-	-	-	=	-	8
Non-infected	-	-	-	=	-	1
Non-infected	-	-	=	-	-	1
Non-infected	-	N/A	-	-	-	1
Non-infected	N/A	-	-	-	+	1
Non-infected	N/A	-	-	-	-	3
Total						640

N/A = specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.

FS = female endocervical swab; **FU** = female urine; **CVS** = clinician-collected vaginal swab; **PVS** = asymptomatic patient-collected vaginal swab.

Table 7d: Clinician-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with C. trachomatis According to Patient Infected Status

Patient Infected Status	NAAT 1 (Aptima Combo 2 Assay)		NAAT 2		Aptima CT Assay	Symptom Status		Total
	FS	FU	FS	FU	CVS	Sym	Asym	
Infected	+	+	+	+	+	76	44	120
Infected	+	+	+	+	-	2	0	2
Infected	+	+	+	+	+	2	0	2
Infected	+	+	+	+	+	1	0	1
Infected	+	+	+	-	+	8	5	13
Infected	+	+	+	-	-	1	0	1
Infected	+	+	+	-	+	1	0	1
Infected	+	+	+	=	+	1	0	1
Infected	+	+	-	+	+	9	3	12
Infected	+	-	+	+	+	5	3	8
Infected	+	-	+	-	+	7	0	7
Infected	-	+	+	+	+	0	1	1
Infected	-	+	-	+	+	1	4	5
Infected	-	+	-	+	-	1	0	1
Infected	-	+	-	+	-	0	1	1
Non-infected	+	+	-	-	+	1	2	3
Non-infected	+	+	-	N/A	+	1	0	1
Non-infected	+	-	-	-	+	3	4	7
Non-infected	+	-	-	-	-	0	1	1
Non-infected	+	-	-	-	+	2	2	4
Non-infected	+	-	-	-	-	5	3	8
Non-infected	+	-	-	-	+	1	0	1
Non-infected	+	-	-	-	-	1	0	1
Non-infected	-	+	-	-	+	5	2	7
Non-infected	-	+	-	-	-	0	2	2
Non-infected	-	-	+	-	-	1	1	2
Non-infected	-	-	-	+	-	1	2	3
Non-infected	-	-	-	-	+	4	5	9
Non-infected	-	-	-	-	-	6	10	16
Non-infected	-	-	-	-	+	16	15	31
Non-infected	-	-	-	-	-	614	500	1114
Non-infected	-	-	-	-	N/A	0	1	1
Non-infected	-	-	-	-	+	0	1	1
Non-infected	-	-	-	-	-	13	9	22
Non-infected	-	-	-	N/A	-	2	2	4
Non-infected	-	-	-	N/A	-	0	1	1
Non-infected	-	-	-	=	+	0	1	1
Non-infected	-	-	-	=	-	12	8	20
Non-infected	-	-	-	=	N/A	0	1	1
Non-infected	-	-	=	-	-	1	1	2
Non-infected	-	N/A	-	-	-	0	1	1

Table 7d: Clinician-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with C. trachomatis According to Patient Infected Status (continued)

Patient Infected Status	NAAT 1 (Aptima Combo 2 Assay)		NAAT 2		Aptima CT Assay	Symptom Status		Total
	FS	FU	FS	FU	CVS	Sym	Asym	
Non-infected	-	N/A	-	-	N/A	1	0	1
Non-infected	N/A	-	-	-	-	0	1	1
Non-infected	N/A	-	-	-	-	5	3	8
Non-infected	=	-	-	-	-	2	0	2
Total						812	640	1452

N/A = specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing. **FS** = female endocervical swab; **FU** = female urine; **CVS** = clinician-collected vaginal swab; **Sym** = symptomatic; **Asym** = asymptomatic.

Table 7e: PreservCyt Solution Liquid Pap Specimen Clinical Study Patient Infected Status Results for C. trachomatis

Patient Infected Status	Endocervical Swab		Symptom Status	
	Aptima Combo 2 Assay	Aptima CT Assay	Symptomatic	Asymptomatic
Infected	Positive	Positive	30	60
Non-Infected	Negative	Negative	322	1214
Non-Infected	Negative	Positive	4	12
Non-Infected	Positive	Negative	3	2
Total			359	1288

RLU Distribution of Aptima Controls

The distribution of the RLUs for the Positive Control, GC / Negative Control, CT and the Positive Control, CT / Negative Control, GC from all the Aptima CT assay runs performed during the clinical specimen studies are presented in Table 8.

Table 8: Distribution of RLU of the Aptima Controls During the Clinical Specimen Studies Including Endocervical, Vaginal and Male Urethral Swab, Male and Female Urine Specimens, and PreservCyt Liquid Pap Studies

Control	Statistics	RLU (x1000)	
		Swab and Urine Specimen Clinical Study	PreservCyt Liquid Pap Specimen Clinical Study
Positive Control, GC / Negative Control, CT	N	198	209
	Mean	0.89	1.22
	SD	2.94	2.63
	Maximum	26	36
	75th Percentile	1	1
	Median	0	1
	25th Percentile	0	1
	Minimum	0	0

Table 8: Distribution of RLU of the Aptima Controls During the Clinical Specimen Studies Including Endocervical, Vaginal and Male Urethral Swab, Male and Female Urine Specimens, and PreservCyt Liquid Pap Studies

Control	Statistics	RLU (x1000)	
		Swab and Urine Specimen Clinical Study	PreservCyt Liquid Pap Specimen Clinical Study
Positive Control, CT / Negative Control, GC	N	198	209
	Mean	7007	6593
	SD	776	709
	Maximum	8884	10383
	75th Percentile	7440	7025
	Median	7066	6661
	25th Percentile	6621	6205
	Minimum	988	4419

Precision Study

Aptima CT assay precision (i.e., reproducibility) was evaluated at two external clinical sites and at Hologic. Aptima CT assay precision was evaluated across three Aptima CT assay kit lots, three study sites, six operators and 108 Aptima CT assay runs. Two operators at each of the three testing sites performed a total of six Aptima CT assay runs per kit lot for a total of 36 runs per kit lot. Each run was composed of a 12-member precision panel containing 0 to 2,000 fg/assay of CT rRNA. Reproducibility was established using spiked swab transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Table 9 presents the precision RLU data in terms of Mean, Standard Deviation, Coefficient of Variation (CV), and percent agreement with expected results for calculations of between-site, between-lot, between-operator, between-run, and within-run variability.

Table 9: Aptima CT Assay Precision Data Using a 12-Member Precision Panel Containing 0 to 2,000 fg/assay of CT rRNA

Concentration	N	Mean RLU (x1000)	% Agrmt.	Within-Run		Between-Site		Between-Lot		Between-Operator		Between-Run	
				SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)
Neg (0 fg/mL)	540	0.7	100	0.7	N/A	0.5	N/A	0.3	N/A	0.4	N/A	0	N/A
Low (12 fg/mL)	216	7143.4	100	200.3	2.8	335.6	4.7	207.7	2.9	537.3	7.5	558.8	7.8
Mid (250 fg/mL)	108	7084.9	100	162.2	2.3	275.1	3.9	159.5	2.3	546.3	7.7	578.2	8.2
Mid (2,500 fg/mL)	108	6991.1	100	150.7	2.2	279.4	4.0	117.8	1.7	532.3	7.6	534.9	7.7
High (5,000–5,135 fg/mL)	324	7133.4	100	229.2	3.2	301.0	4.2	129.0	1.8	531.7	7.5	618.3	8.7

SD = standard deviation; **CV(%)** = percent coefficient of variation; **% Agrmt.** = percent agreement.

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and %CV is set to zero (17).

PreservCyt specimen within-laboratory precision with the Aptima CT assay was determined by spiking PreservCyt vials with 20 CT IFU per vial (0.1 IFU per reaction) and 100 CT IFU per vial (0.5 IFU per reaction). Vials containing 1,000 CT IFU per vial (5 IFU per reaction) and unspiked PreservCyt vials were tested as positive and negative controls. Ten vials spiked at each IFU level and ten unspiked vials were divided between two operators. The

operators vortexed the vials and then transferred 14 aliquots (1.0 mL each) per vial into 14 Aptima transfer tubes as per the Aptima Specimen Transfer Kit package insert. The operators were blinded to the samples' titers. Each of the resulting Pap-STM samples was tested once in the Aptima CT assay. A total of five runs were performed over a five day period for 140 results at each IFU level. The results are summarized in Table 10.

Table 10: Aptima CT Assay Within-Laboratory Precision Data for PreservCyt using a 4-Member Precision Panel containing 0 to 1000 IFU/20 mL of CT cells

Panel Member	IFU/20mL PreservCyt	IFU/ rxn	n	Agreed	% Agrmt.	Mean RLU (x1000)	Within-Operator		Between-Day		Between-Operator		Total	
							SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
A	20	0.1	140	140	100	6501.7	734.8	11.3	0	0.0	546.9	8.4	916	14.1
B	100	0.5	140	138*	98.6	6337.7	1054.7	16.6	0	0.0	947.2	14.9	1417.6	22.4
C	1000	5	140	140	100	6521.9	909	13.9	247.1	3.8	393.9	6	1021	15.7
D	0	0	140	140	100	1.2	0.8	N/A	0	N/A	0.4	N/A	0.9	N/A

SD = standard deviation; **CV (%)** = percent coefficient of variation; **% Agrmt.** = percent agreement; **N/A** = not applicable; **RLU** = relative light unit; **Operator** = run.

* Discordant results were one negative result and 1 equivocal result.

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and %CV is set to zero (17). Samples with discordant results were included in the signal variability analysis.

DTS Systems Analytical Performance

See *Tigris DTS System Analytical Performance* following the *Tigris DTS System Clinical Specimen Agreement* section for Tigris DTS system-specific analytical performance.

See *Panther System Analytical Performance* for Panther system-specific analytical performance.

Analytical Sensitivity

C. trachomatis analytical sensitivity (limit of detection) was determined by directly comparing dilutions of CT organisms in cell culture and in the Aptima CT assay. The analytical sensitivity claim for the assay is one Inclusion-Forming Unit (IFU) per assay (7.25 IFU/swab, 5 IFU/mL urine, and 9.75 IFU/mL PreservCyt solution liquid Pap specimen) for all 15 CT serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2 and L3). However, dilutions of less than one IFU/assay of all serovars tested positive.

Analytical Specificity

A total of 154 culture isolates were evaluated using the Aptima CT assay. 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 in KOVA-Trol/Urine Transport Media (UTM) and 60 organisms were tested in STM. The Chlamydia and Neisseria organisms were tested in the PreservCyt solution media. *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 11.

Table 11: 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.

Interfering Substances

The following interfering substances were individually spiked into swab, PreservCyt solution liquid Pap and/or urine specimens: 10% blood, 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: 30% blood, 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.

Recovery

Escherichia coli, Gardnerella vaginalis, *Lactobacillus acidophilus*, *Bacteroides ureolyticus,* and *Staphylococcus epidermidis* (1 x 10^8 cells/assay) were added to samples containing the rRNA equivalent of approximately one CT IFU (5 fg). These additions did not interfere with the amplification and detection of CT rRNA using the Aptima CT assay.

Specimen Stability Studies

A. Swab Specimens

Data to support the recommended shipping and storage conditions for endocervical, urethral and vaginal swab samples were generated with pooled negative swab samples. Pooled samples were spiked with CT at a final concentration of 1 IFU per reaction. The spiked samples were held at –70°C, –20°C, 4°C, and 30°C. Samples were tested in duplicate at days 0, 20, 77, and 117. All test conditions were positive for CT at all times and temperatures.

B. Urine Specimens

Data to support the recommended shipping and storage conditions for urine samples were generated with 10 female and 10 male negative urine samples. The urine samples were spiked with CT at a final concentration of 10 IFU per reaction. Two sets of the spiked urine samples were held at 4°C and 30°C prior to being added to the UTM. The two sets of UTM samples then were held at 4°C and 30°C and tested in triplicate at days 0, 1, 5, 20, and 35. All samples met the pre-specified acceptance criteria at day 35.

C. PreservCyt Solution Liquid Pap Specimens

Data to support the recommended shipping and storage conditions for PreservCyt solution liquid Pap samples were generated with negative processed and unprocessed liquid Pap samples. For the unprocessed samples, four pools of PreservCyt solution samples were tested after being stored in the PreservCyt solution vial. Each specimen pool was spiked with 1 to 10 IFU CT/assay, held at 2°C, 10°C, and 30°C, then tested at baseline and on days 5, 7, 8, 14, 18, 21, 25 and 36. All of the spiked samples were positive for CT at all times and temperatures.

For the processed samples, four pools of PreservCyt solution samples were used to determine processed specimen stability at 2°C to 30°C. Each negative sample pool was spiked with 1 to 10 IFU CT/assay, then tested at baseline. Prior to processing, the PreservCyt solution samples were stored at 30°C for seven (7) days to simulate the time

lapse between sample collection, Pap processing and shipment to a microbiology testing lab. After seven days at 30°C, 1 mL aliquots of each pool were transferred to an Aptima specimen transfer tube and tested at baseline before being placed at 2°C, 10°C, and 30°C. The processed samples were then tested for 17 days stored at 30°C and 36 days stored at 2°C to 10°C. All of the spiked samples were positive for CT at all times and temperatures.

Data to support longer storage conditions were generated from four pools of negative processed PreservCyt solution samples tested at below freezing temperatures. Each pool was spiked with 1 to 10 IFU CT/assay, then tested at baseline. Each pool was first placed at 30°C for 14 days and then stored at –20°C or –70°C over the course of 106 days. All of the spiked samples were positive for CT at all times and temperatures.

D. Additional Frozen (at –20°C) Specimen Stability Study

The recommended frozen storage conditions for endocervical swab, urethral swab, vaginal swab, female urine, male urine, and PreservCyt solution liquid Pap specimens in transport media is between –20°C and –70°C to allow testing up to 12 months. Supporting data for each specimen type were generated using 90 negative specimens. Of these, 30 specimens were spiked with CT at 1.0 IFU per reaction; 30 specimens were spiked at 0.1 IFU per reaction; and 30 specimens were not spiked. The specimens in transport media were stored frozen within 7 days of collection and tested at days 200 and 400. Specimens met the acceptance criteria of 95% agreement with expected results.

Tigris DTS System Clinical Specimen Agreement

Tigris DTS System Agreement

Agreement between Aptima CT assay results generated on the fully automated Tigris DTS system and semi-automated DTS systems was evaluated by testing endocervical swab, male urethral swab, male and female urine, vaginal swab, and PreservCyt solution liquid Pap specimens. Each of the clinical specimens was tested individually with the Aptima CT assay on both the Tigris DTS system and DTS systems at Hologic. The order of testing was not randomized. Specimens identified for inclusion were tested on the Tigris DTS system followed by testing on DTS systems.

Clinical Specimen Agreement Study — Endocervical Swab, Male Urethral Swab, Female and Male Urine, Vaginal Swab, and PreservCyt Liquid Pap Specimens

Female and male subjects attending STD, family planning, and OB/GYN clinics from eight geographically diverse sites with low to high prevalence for CT contributed endocervical swab, male urethral swab, female and male urine, vaginal swab, and PreservCyt solution liquid Pap specimens. The specimens were transferred directly to Hologic for testing while the PreservCyt solution liquid Pap specimens were processed at 2 cytopathology laboratories before being transferred. At Hologic, endocervical swab, male urethral swab, female and male urine specimens were first screened with Aptima Combo 2 assay on the Tigris DTS system, and the vaginal swab and PreservCyt solution liquid Pap specimens were screened with Aptima Combo 2 assay on the DTS systems. Specimens with final invalid or equivocal results were not selected in the Aptima CT Clinical Specimen Agreement Study.

Two hundred and five female swabs (87 endocervical and 118 vaginal), 120 male urethral swab, 98 female urine, 115 male urine, and 116 PreservCyt solution liquid Pap specimens with Aptima Combo 2 assay CT positive and negative results were selected for comparison testing between the Tigris DTS system and the DTS systems for the Aptima CT assay. Specimens with initial invalid or equivocal results were retested using the same system on which the result was generated. One female urine specimen had an initial equivocal result on the DTS systems; when retested, the final result was valid. One male urine specimen had an initial invalid result on the Tigris DTS system; when retested, the final result was valid. One female urine specimen had an initial equivocal result on the Tigris DTS system; this specimen was retested, however, the specimen had expired, so the final result was equivocal.

Table 12 shows the positive, negative, and overall agreements for all paired results for each specimen type by symptomatic status. Specimens are relatively imbalanced by symptomatic and asymptomatic status but overall agreements for symptomatic subjects were 98.5% (131/133) for female swabs (combined endocervical and vaginal swabs), 100% (60/60) for male urethral swab, 98.2% (55/56) for female urine specimens, 100% (60/60) for male urine specimens, and 100% (81/81) for PreservCyt solution liquid Pap specimens. For asymptomatic subjects, overall agreements were 100% for 72 female swabs, 60 male urethral swabs, 42 female urine, 55 male urine specimens, and 35 PreservCyt solution liquid Pap specimens, respectively. For ‘All’ (symptomatic and asymptomatic combined) subjects, overall agreement was 99.0% (203/205) for female swab (combined endocervical and vaginal swabs), 100% (120/120) for male urethral swab, 99.0% (97/98) for female urine, 100% (115/115) for male urine, and 100% (116/116) for PreservCyt solution liquid Pap specimens. Due to the relatively smaller specimen number from asymptomatic subjects, these findings may not be generalizable to Aptima CT-Tigris system testing with specimens from asymptomatic subjects.

Refer to Tables 4 and 5a for Aptima CT assay sensitivity and specificity estimates from testing on the DTS systems. Sensitivity and specificity of the Aptima CT assay when using the Tigris DTS system would be expected to be similar given the agreement findings.

Table 12: Clinical Specimen Agreement Study: Positive, Negative, and Overall Agreements by Symptom Status

Symptom	Specimen	Gender	n	DTS+ Tigris+	DTS+ Tigris-	DTS- Tigris+	DTS- Tigris-	Positive % Agreement (95% CI)	Negative % Agreement (95% CI)	Overall % Agreement (95% CI)
Sympt.	Swab	Female*	133	63	1	1	68	98.4 (91.6–100)	98.6 (92.2–100)	98.5 (94.7–99.8)
		Male	60	42	0	0	18	100 (91.6–100)	100 (81.5–100)	100 (94.0–100)
	Urine	Female	56	33	0	1[1]	22	100 (89.4–100)	95.7 (78.1–99.9)	98.2 (90.4–100)
		Male	60	41	0	0	19	100 (91.4–100)	100 (82.4–100)	100 (94.0–100)
	PreservCyt Solution	Female	81	39	0	0	42	100 (91.0–100)	100 (91.6–100)	100 (95.5–100)
Asympt.	Swab	Female*	72	41	0	0	31	100 (91.4–100)	100 (88.8–100)	100 (95.0–100)
		Male	60	23	0	0	37	100 (85.2–100)	100 (90.5–100)	100 (94.0–100)
	Urine	Female	42	23	0	0	19	100 (85.2–100)	100 (82.4–100)	100 (91.6–100)
		Male	55	20	0	0	35	100 (83.2–100)	100 (90.0–100)	100 (93.5–100)
	PreservCyt Solution	Female	35	25	0	0	10	100 (86.3–100)	100 (69.2–100)	100 (90.0–100)
All	Swab	Female*	205	104	1	1	99	99.0 (94.8–100)	99.0 (94.6–100)	99.0 (96.5–99.9)
		Male	120	65	0	0	55	100 (94.5–100)	100 (93.5–100)	100 (97.0–100)
	Urine	Female	98	56	0	1[1]	41	100 (93.6–100)	97.6 (87.4–99.9)	99.0 (94.4–100)
		Male	115	61	0	0	54	100 (94.1–100)	100 (93.4–100)	100 (96.8–100)
	PreservCyt Solution	Female	116	64	0	0	52	100 (94.4–100)	100 (93.2–100)	100 (96.9–100)

"+" denotes a positive result; "-" a negative result; **CI** = confidence interval.

*Endocervical and Vaginal Swab samples combined.

[1]Specimen had a final equivocal result on the Tigris DTS System.

Precision Study

The effect of several factors on the variability of Aptima CT assay performance on the Tigris DTS system was evaluated using 12-member STD reproducibility panels. Panel members contained 0 to 5,000 fg CT rRNA/assay. The panel included panel members with CT concentrations at the analytical sensitivity claim of 5 fg CT rRNA/assay.

The panels were tested at one external testing site and at Hologic using two Aptima CT assay reagent lots. At Hologic, two operators each performed three valid worklists per reagent lot on each of two Tigris DTS system instruments. At the external testing site, two operators each performed three valid worklists per reagent lot on one Tigris DTS system instrument. One worklist consisted of run controls and six 12-member panels.

Reproducibility was determined by calculating the agreement between the final assay results and the expected outcome for each panel member. Reproducibility was also assessed by calculating the SD and coefficient of variation (CV) of signal with respect to sites, operators, lots, and worklists. CVs were not calculated for CT-negative panel members due to low signal values that could theoretically equal zero. Table 13 shows the reproducibility results. All Aptima CT assay results on the Tigris DTS system agreed with the expected results. CV values were less than or equal to 3.4%. These data indicate excellent reproducibility of the Aptima CT assay using the Tigris DTS system.

Table 13: Tigris DTS System Precision Data

Conc (fg rRNA per assay)	n	Mean RLU (x1000)	% Agrmt	Between-Site		Between-Operator		Between-Lot		Between-Worklist		Within-Worklist	
				SD[1] (x1000)	CV[1] (%)	SD (x1000)	CV (%)	SD[1] (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
0	863	2.9	100	1.4	N/A	0.3	N/A	0.0	N/A	0.2	N/A	2.2	N/A
5	432	7041	100	32.0	0.5	217	3.1	63.7	0.9	174	2.5	206	2.9
50	433[2]	7090	100	0.0	0.0	224	3.2	93.1	1.3	168	2.4	189	2.7
500	431[3]	7130	100	0.0	0.0	240	3.4	96.9	1.4	164	2.3	217	3.0
5,000	432	7152	100	0.0	0.0	208	2.9	85.7	1.2	179	2.5	211	3.0

Agrmt = agreement; **Conc** = concentration; **CV** = coefficient of variation; **N/A** = not applicable for negative samples; **RLU** = relative light unit; **SD** = standard deviation.

[1] SD and CV values are set to 0 and 0.0%, respectively, according to the random effects model, if the variability due to this source relative to random errors and/or variation of other sources is numerically negative.

[2] One worklist included 1 additional replicate of a panel member with 50 fg rRNA/assay.

[3] One worklist was missing 1 replicate of a panel member with 500 fg rRNA/assay.

Tigris DTS System Analytical Performance

See *Panther System Analytical Performance* for Panther system-specific analytical performance.

Analytical Sensitivity Equivalence Study

Sensitivity panels in endocervical swab pool, vaginal specimen pool, urine specimen pool, and PreservCyt solution liquid Pap specimen pool were prepared at CT rRNA equivalent of 1 IFU per assay (7.25 IFU/swab and 5 IFU/mL urine) and tested 60 replicates on the Tigris DTS system. Percent positivity (95% CI) on the Tigris DTS system for endocervical swab specimen was 100% (95.1–100), for vaginal swab specimen was 100% (95.1–100), for urine specimen was 100% (95.1–100), and for PreservCyt solution liquid Pap specimen was 100% (95.1–100).

CT rRNA Spiked Clinical Panel Study

The CT rRNA spiked clinical panel study evaluated agreement between the two systems (Tigris DTS system and DTS systems) using six Hologic prepared CT clinical panels spiked with 0 to 5,000 fg rRNA/assay of CT. The CT clinical panels were created from endocervical swab, vaginal swab, urethral swab, male urine, female urine, and PreservCyt solution liquid Pap specimens that had negative Aptima CT results on the DTS systems when tested at Hologic. The negative specimens were pooled by specimen type, spiked or not spiked with CT rRNA and aliquotted as replicates of each panel member. Replicates of each of 6-panel members with different spiked rRNA levels were combined to create one clinical panel for each specimen type. Each panel contained a total of 132 replicates.

Table 14 shows the percent agreement for each level of rRNA in the endocervical swab, vaginal swab, urethral swab, male urine, female urine, and PreservCyt solution liquid Pap panels, respectively, with expected CT results for the Tigris DTS system and for the DTS systems. The concentration ranged from 1 log below to 3 logs above the 5 fg rRNA/assay for CT. Also shown in Table 14 are the overall percent agreements of the clinical panel study between the Tigris DTS system and DTS systems.

Table 14: CT rRNA Spiked Clinical Panel Agreement Study

Specimen		Panel Member	Concentration (fg rRNA/Assay)	Replicates	Tigris % Agreement	DTS % Agreement	Overall % Agreement between Tigris and DTS (95% CI)
Swab	Endocervical	No Target	0	12	100	100	100 (97.2–100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
	Vaginal	No Target	0	12	100	100	100 (97.2–100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
	Urethral	No Target	0	12	100	100	100 (97.2–100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
Urine	Male	No Target	0	12	91.7 (11/12)	100	99.2 (95.9–100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
	Female	No Target	0	12	100	100	100 (97.2–100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
PreservCyt Solution Liquid Pap		No Target	0	12	100	100	100 (97.2–100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	

CI = confidence interval.

Analytical Specificity Equivalence Study

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 Tigris DTS system and the DTS systems, analytical specificity experiments on the Tigris DTS system were designed to focus on the most challenging culture isolates. These organisms included those known to cross-react in other amplification assays. Twenty-four (24) culture isolates were selected from the panel of organisms in

Table 11, including 3 organisms that are most closely related to CT. All of the organisms tested produced negative results on the Tigris DTS system.

Interfering Substances Equivalence Study

Whole blood, a substance commonly found in urogenital specimens and known to interfere in some amplification assays, was used to establish that the Tigris DTS system tolerates similar levels of potentially interfering substances as does the DTS systems. Fresh blood was added to clinical swab, vaginal swab, urine, and PreservCyt solution liquid Pap specimen pools, then tested for potential assay interference in the absence and presence of CT target at the estimated rRNA equivalent of one CT IFU/assay (5 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. Specimens were tested on two Tigris DTS systems. All samples containing target nucleic acid were positive when tested at a level of 10% blood in swab specimens, vaginal swab specimens, PreservCyt solution liquid Pap specimens, and 30% blood in urine specimens. All samples that did not contain target were negative for CT. These results indicate that at the levels tested, whole blood is unlikely to affect the CT result on the Tigris DTS system.

Carryover Studies for the Tigris DTS System

To establish that the Tigris DTS system minimizes the risk of false positive results arising from carryover contamination, a study was conducted using spiked panels on three Tigris DTS systems. The study used 20% high-target samples containing 1 x 10^6 fg CT rRNA/mL, which were randomly spaced amongst 80% negative samples containing STM. In the study, 576 high-target samples and 2,376 negative samples were tested across the three Tigris DTS systems. Table 15 shows the overall carryover rate was averaged at 0.21% (5/2364). A total of 12 negative samples were reported as invalid and were excluded from the calculation. A separate analysis was conducted on a subset of the study population comprised of the negative samples that immediately followed a high-target positive. The carryover rate for this subset of the population was averaged at 0.47% (2/424). For false positives in this subset, the carryover rate ranged from 0% to 1.43% across the three Tigris DTS systems. These results demonstrate that carryover contamination is minimized on the Tigris DTS system.

Table 15: Summary of Overall Tigris DTS System Carryover

Instrument	# Valid Negative Tests	Total # CT False Positive Results	% CT False Positive Results	Confidence Intervals (95% CI)
Tigris 1	789	2[a]	0.25	0.03–0.91
Tigris 2	783	3[b]	0.38	0.08–1.12
Tigris 3	792	0[c]	0.00	0.00–0.38
All Instruments	2364	5	0.21	0.07–0.49

CI = confidence interval.

[a.] Tigris 1 had no false CT positive result directly following a high-target positive.

[b.] Tigris 2 had two false CT positive results directly following a high-target positive.

[c.] Tigris 3 had no false CT positive result directly following a high-target positive.

Panther System Analytical Performance

Spiked Clinical Panel Agreement Study

Individual negative urine specimens were spiked with CT serovar G to create a panel of 120 CT positives. CT positive panel members were spiked with organisms at 0.25 IFU/mL, 2.5 IFU/mL or 25 IFU/mL (0.5 fg/assay, 5 fg/assay or 50 fg/assay). In addition, 120 CT negative urine specimens were collected. The positive and negative panels were tested on three Panther and three Tigris DTS systems. Positive percent agreement between the Panther system and the Tigris DTS system was 100% with a lower 95% confidence interval of 98.9 for CT. Negative percent agreement between the Panther system and the Tigris DTS systems was 100% with a lower 95% confidence interval of 98.9. The results of the study are shown in Table 16.

Table 16: Spiked Clinical Panel Agreement Study: Agreement with Expected CT Results

Panel Member	Concentration		Replicates	Tigris %Agrmt	Panther %Agrmt
	IFU/mL	fg/assay			
Very Low Positive	0.25	0.5	120	100	100
Low Positive	2.5	5	120	100	100
Medium Positive	25	50	120	100	100
Negative	0	0	360	100	100

% Agrmt = percent agreement.
Overall Positive Percent Agreement between Tigris and Panther (95% CI): 100% (98.9–100).
Overall Negative Percent Agreement between Tigris and Panther (95% CI): 100% (98.9–100).

Analytical Sensitivity Study

Analytical sensitivity of the Aptima CT assay was tested using three representative sample matrices. These were urine processed with UTM, PreservCyt liquid Pap solution diluted with STM, and STM. CT rRNA was spiked into pools of these three matrices at the following concentrations 0.5 fg/assay, 5 fg/assay and 50 fg/assay (rRNA equivalents of 0.25 IFU/mL, 2.5 IFU/mL or 25 IFU/mL). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. These panels were tested on three Panther systems using two lots of reagents in replicates of 96. Positive agreement with the expected result was calculated. Agreement to expected results was 100% (95% CI 96.2–100%) for all urine panels, 100% (95% CI 96.1–100%) for all PreservCyt solution liquid Pap specimen panels, and 100% for the (95% CI 96.0–100%) for all STM panels. The analytical sensitivity for the assay is 2.5 IFU/mL.

Reproducibility Study

The Aptima CT assay precision was evaluated across three Panther systems and two Aptima CT assay kit lots over a period of 24 days. Panels were made by spiking CT rRNA into STM at the concentrations shown in Table 17. Operators performed two runs per day running each panel member in replicates of two per run. The agreement with the expected result was calculated and precision was estimated according to NCCLS Guidelines EP5-A2 (19). The total number of replicates for each panel was 93–96. Table 17 presents the precision RLU data in terms of Mean, Standard Deviation, Coefficient of Variation (CV), percent agreement with expected results and calculations of between-instrument, between-lot, between-run, and within-run variability.

Table 17: Panther Precision for Aptima CT assay

Matrix	CT (IFU/mL)	N*	Mean RLU (x1000)	% Agrmt	Between-instrument		Between-lot		Between-Run		Within-Run		Total	
					SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
STM	0	96	2	100	0.38	21.3	0.64	35.8	0	0	1.86	104.6	2	112.3
	0.25	93	7390	100	221.74	3	264.35	3.6	0	0	180.07	2.4	389.2	5.3
	2.5	96	7478	100	224.45	3	249.88	3.3	53.1	0.7	164.57	2.2	377.8	5.1
	25	96	7482	100	222.23	3	233.36	3.1	46.47	0.6	180.29	2.4	372.2	5
Urine	0	95	2	100	0.23	12.7	0.38	20.7	0.52	28.5	1.3	71	1.5	81.9
	0.25	96	6978	100	276.94	4	330.57	4.7	66.36	1	264.73	3.8	510.4	7.3
	2.5	95	7291	100	121.2	1.7	154.63	2.1	73.51	1	148.13	2	256.8	3.5
	25	95	7349	100	121.57	1.7	181.34	2.5	66.87	0.9	162.45	2.2	280.2	3.8
PreservCyt	0	96	7	97.9	3.36	46.1	0.29	4	0	0	20.52	281.4	20.8	285.3
	0.25	96	6996	100	225.16	3.2	209.86	3	0	0	164.87	2.4	349.2	5
	2.5	95	7079	100	246.89	3.5	172.55	2.4	0	0	151.67	2.1	337.2	4.8
	25	96	7050	100	262.52	3.7	167.79	2.4	0	0	192.5	2.7	366.2	5.2

% Agrmt = %agreement; **CV** = coefficient of variation; **N** = number of panel members; **SD** = standard deviation; **RLU** = relative light unit.

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

* Total number of replicates for each panel = 96. In select runs, individual invalid replicates were not retested.

Analytical Specificity

Analytical specificity was not tested on the Panther instrument. Please refer to *Tigris DTS System Analytical Performance* for *Analytical Specificity Equivalence Study*.

Interfering Substances Equivalence Study

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, post-processed PreservCyt solution Liquid Pap specimens or 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 or PreservCyt solution liquid Pap specimens, or 30% (vol/vol) blood in urine specimens. All samples that did not contain target were correctly identified as negative. These results are identical to those demonstrated for the Tigris DTS system when spiked with the same quantities of blood. Blood added to swab, PreservCyt solution, 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

To establish that the Panther system minimizes the risk of false positive results arising from carryover contamination, a multi-run analytical study was conducted using spiked panels on three 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 in a specific pattern within the run. High titer samples were made using CT rRNA spiked into STM to give a final concentration of 5×10^5 fg rRNA/reaction (rRNA equivalent of 2.5×10^5 IFU/mL). Testing was carried out using 5 runs on three Panther systems with a total of 2933 negative samples. The overall carryover rate was 0% with a 95% confidence interval of 0–0.1%. A total of 7 negative samples were reported as invalid in the high titer carryover runs and were excluded from the calculation.

Bibliography

1. **Beem, M. O., and E. M. Saxon**. 1977. Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. NEJM **296**:306-310.
2. **Buimer, M., G. J. J. Van Doornum, S. Ching, P. G. H. Peerbooms, P. K. Plier, D. Ram, and H. H. Lee**. 1996. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Ligase chain reaction-based assays with clinical specimens from various sites: implications for diagnostic testing and screening. J. Clin. Microbiol. **34**:2395-2400.
3. **Cates, Jr., W., and J. N. Wasserheit**. 1991. Genital chlamydia infections: epidemiology and reproductive sequelae. Am. J. Obstet. Gynecol. **164**:1771-1781.
4. **Centers for Disease Control and Prevention.** 2002. Screening Tests to Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. United States Morbid. and Mortal. Weekly Rep. **51** (RR-15).
5. **Centers for Disease Control and Prevention.** Sexually Transmitted Disease Surveillance 2019. Last reviewed April 13, 2021. Accessed May 6, 2021. https://www.cdc.gov/std/statistics/2019/overview.htm
6. **Chernesky, M. A., D. Jang, J. Sellors, K. Luinstra, S. Chong, S. Castriciano, and J. B. Mahony**. 1996. Urinary inhibitors of polymerase chain reaction and Ligase chain reaction and testing of multiple specimens may contribute to lower assay sensitivities for diagnosing *Chlamydia trachomatis* infected women. Mol. Cell. Probes. **11**:243-249.
7. **Chong, S., D. Jang, X. Song, J. Mahony, A. Petrich, P. Barriga, and M. Chernesky.** 2003. Specimen processing and concentration of *Chlamydia trachomatis* added can influence false-negative rates in the LCx assay but not in the APTIMA Combo 2 Assay when testing for inhibitors. J. Clin. Microbiol. **41**:778-782.
8. **Crotchfelt, K. A., B. Pare, C. Gaydos, and T. C. Quinn.** 1998. Detection of *Chlamydia trachomatis* by the Hologic AMPLIFIED Chlamydia Trachomatis assay (AMP CT) in urine specimens from men and women and endocervical specimens from women. J. Clin. Microbiol. **36**:391-394.
9. **CUMITECH 31**. Verification and Validation of Procedures in the Clinical Microbiology Laboratory.- ASM PRESS, FEBRUARY 1997.
10. **Frommell, G. T., R. Rothenberg, S. Wang, and K. McIntosh**. 1979. Chlamydial infection of mothers and their infants. Journal of Pediatrics **95**:28-32.
11. **Gaydos, C. A., T.C. Quinn, D. Willis, A. Weissfeld, E. W. Hook, D. H. Martin, D. V. Ferraro, and J. Schachter.** 2003. Performance of the APTIMA Combo 2 Assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. J. Clin. Microbiol. **41**:304-309.
12. **Goessens, W. H. F., J. W. Mouton, W. I. Van Der Meijden, S. Deelen, T. H. Van Rijsoort-Vos, N. L. Toom, H. Verbrugh, and R. P. Verkooyen.** 1997. Comparison of three commercially available amplification assays, AMP CT, LCx, and COBAS AMPLICOR, for detection of *Chlamydia trachomatis* in first-void urine. J. Clin. Microbiol. **35**:2628-2633.
13. **Holmes, K. K., H. H. Handsfield, S. P. Wang, B. B. Wentworth, M. Turck, J. B. Anderson, and E. R. Alexander**. 1975. Etiology of nongonococcal urethritis. NEJM **292**:1199-1205.
14. **Jaschek, G., C. A. Gaydos, L. E. Welsh, and T. C. Quinn.** 1993. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. J. Clin. Microbiol. **31**:1209-1212.
15. **Mahony, J., S. Chong, D. Jang, K. Luinstra, M. Faught, D. Dalby, J. Sellors, and M. Chernesky**. 1998. Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, Ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. J. Clin. Microbiol. **36**:3122-3126.
16. **McCurdy, Brenda W.** 1997. Cumitech Guide on Verification and Validation of Procedures in the Microbiology Laboratory. February, 1997, American Society for Microbiology. ASM Press.
17. **National Committee for Clinical Laboratory Standards.** 1999. NCCLS EP5-A: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (Vol. 19, No. 2).
18. **National Committee for Clinical Laboratory Standards.** 2002. User Protocol for Evaluation of Qualitative Test Performance: Approved Guideline for additional Guidance on Appropriate Internal Quality Control Testing Practices.
19. **National Committee for Clinical Laboratory Standards**. 2004. NCCLS EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline (2nd edition, Vol. 24, No. 25).
20. **Peterson E. M., V. Darrow, J. Blanding, S. Aarnaes, and L. M. de La Maza.** 1997. Reproducibility problems with the AMPLICOR PCR *Chlamydia trachomatis* test, J. Clin. Microbiol. **35**:957-959.
21. **Schachter, J.** 1985. Chlamydiae (Psittacosis-Lymphogranuloma Venereum-Trachoma group), p. 856-862. *In* E. H. Lennette, et al. (ed.), Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
22. **Schachter, J., and M. Grossman.** 1981. chlamydial infections. Ann. Rev. Med. **32**:45-61.
23. **Schachter, J.** 1978. Medical progress: chlamydial infections (third of three parts). NEJM **298**:540-549.
24. **Schachter, J., E. C. Hill, E. B. King, V. R. Coleman, P. Jones, and K. F. Meyer.** 1975. Chlamydial infection in women with cervical dysplasia. Am. J. Obstet. Gynecol. **123**:753-757.
25. **Stary, A., E. Schuh, M. Kerschbaumer, B. Gotz, and H. Lee**. 1998. Performance of transcription-mediated amplification and Ligase chain reaction assays for detection of chlamydial infection in urogenital samples obtained by invasive and noninvasive methods. J. Clin. Microbiol. **36**:2666-2670.
26. **Toye, B., W. Woods, M. Bobrowska, and K. Ramotar.** 1998. Inhibition of PCR in genital and urine specimens submitted for *Chlamydia trachomatis* testing. J. Clin. Microbiol. **36**:2356-2358.

27. **Verkooyen, R. P., A. Luijendijk, W. M. Huisman, W. H. F. Goessens, J. A. J. W. Kluytmans, J. H. Rijsoort-Vos, and H. A. Verbrugh.** 1996. Detection of PCR inhibitors in cervical specimens by using the AMPLICOR *Chlamydia trachomatis a*ssay. J. Clin. Microbiol. **34**:3072-3074.
28. **Vincelette, J., J. Schirm, M. Bogard, A. Bourgault, D. Luijt, A. Bianchi, P. C. Van Voorst Vader, A. Butcher, and M. Rosenstraus.** 1999. Multicenter evaluation of the fully automated COBAS AMPLICOR PCR test for detection of *Chlamydia trachomatis* in urogenital specimens. J. Clin. Microbiol. **3**:74-80.
29. **Yuan, Y., Y-X. Zhang, N. G. Watkins, and H. D. Caldwell.** 1989. Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 *Chlamydia trachomatis* serovars. Infect. Immun. **57**:1040-1049.

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

IVD CE 1434

EC REP
Hologic BV, Da Vincilaan 5
1930 Zaventem, Belgium

Hologic (Australia & New Zealand) Pty Ltd
Macquarie Park, NSW 2113

For country-specific Technical Support and Customer Service email address and telephone number, visit www.hologic.com/support.

502184EN Rev. 011
2024-01