Aptima Combo 2™ Assay

For *in vitro* diagnostic use.

For U.S. Export only.

General Information

Intended Use

The Aptima Combo 2™ assay is a target amplification nucleic acid probe test that utilizes target capture for the *in vitro* qualitative detection and differentiation of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) to aid in the diagnosis of chlamydial and/or gonococcal disease using the Tigris™ DTS™ system or Panther™ system, as specified. The assay may be used to test the following specimens from both symptomatic and asymptomatic individuals: clinician-collected endocervical, vaginal, male urethral, and both male and female throat and rectal swab specimens; patient-collected vaginal, both male and female throat and rectal swab specimens[1], and female and male urine specimens. The 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 and the ThinPrep™ 5000 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 (CT) and *Neisseria gonorrhoeae* (GC) infections are two of the most common sexually transmitted infections worldwide. In the United States alone, a total of 1,758,668 cases of CT infections (539.9 per 100,000 population) and 583,405 cases of GC infections (179.1 per 100,000 population) were reported to the Centers for Disease Control in 2018 (9).

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 (59). The serovars D through K are the major cause of genital chlamydial infections in men and women (44). *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and Pelvic Inflammatory Disease (PID) (7, 24, 46, 47). *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, 17, 45).

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 test (NAAT) DNA probe assays. Cell culture was once considered to be the “gold standard” for detection of CT. Culture is quite specific, but scientific publications have demonstrated that the NAAT DNA probe technologies have a higher clinical sensitivity than culture (6, 14, 26, 50). Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe and NAATs.

N. gonorrhoeae is the causative agent of gonorrheal disease. *N. gonorrhoeae* are non-motile, gram-negative diplococci. The majority of gonorrheal infections are uncomplicated lower genital tract infections and may be asymptomatic. However, if left untreated in women, infections can ascend and cause PID. PID can manifest as endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscesses. In men, gonorrhea may be complicated by epididymitis. In rare cases, this may lead to infertility (5). A smaller percentage of persons with gonococcal infections may develop Disseminated Gonococcal Infection (DGI) (23, 32).

Conventional diagnosis of GC infection requires isolation of the organism on selective media or the observation of diplococci in Gram stained smears (25). Culture methods can have good clinical sensitivity, but are highly dependent on proper specimen handling. Improper specimen storage and transport can result in the loss of organism viability and yield false negative results. In addition, poor sampling technique, toxic sampling materials, and the inhibition of growth by components of body secretions can also result in false negative results (11, 28). Non-culture methods for GC detection include direct DNA probe tests and NAATs.

First generation NAATs for CT and GC have technological issues that have limited their performance. These issues include cumbersome specimen processing and specimen inhibition that can yield false negative results (10, 15, 20, 30, 41, 51, 57, 58). The Aptima Combo 2 assay is a second generation NAAT that utilizes target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) 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 DKA technologies (12, 18). The Aptima Combo 2 assay qualitatively detects CT and/or GC rRNA in clinician-collected endocervical, PreservCyt Solution liquid Pap specimens, vaginal, male urethral, and both male and female throat and rectal swab specimens; patient-collected vaginal, and both male and female throat and rectal swab specimens, and female and male urine specimens from symptomatic and asymptomatic individuals.

In 2019, novel *C. trachomatis* variants were discovered which contain point mutations affecting detection by the original version of the Aptima Combo 2 assay (22, 27, 42, 43, 55, 56). Variant strains of chlamydia with mutations affecting diagnostic test performance have been reported previously (54) and are a natural product of microbial evolution. The updated version of the Aptima Combo 2 assay provides detection coverage for the variant strains of *C. trachomatis* that emerged in 2019.

Principles of the Procedure

The Aptima Combo 2 assay combines the technologies of target capture, TMA, and DKA. Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the rRNA targets and protect them from degradation during storage. When the Aptima Combo 2 assay is performed in the laboratory, the target rRNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. 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 molecules 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 Aptima Combo 2 Assay replicates a specific region of the 23S rRNA from CT

and a specific region of the 16S rRNA from GC via DNA intermediates. A unique set of primers is used for each target molecule. Detection of the rRNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent DNA probes, which are complementary to a region of each target amplicon, are labeled with different acridinium ester molecules. The labeled DNA probes combine 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). In DKA, differences in the kinetic profiles of the CT and GC labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for CT signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for GC signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

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

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

Laboratory Related

E. The assay was not evaluated in patient populations with a low prevalence of CT disease; therefore, performance in low prevalence settings has not been determined.

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: Irritants and Corrosives**: Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash with water. If spills of these fluids occur, dilute with water before wiping dry.

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

Specimen Related

J. This assay has been tested using clinician-collected endocervical, PreservCyt Solution liquid Pap specimens, vaginal, male urethral, and both male and female throat and rectal swab specimens; patient-collected vaginal, and both male and female throat and rectal swab specimens, and female and male urine specimens. Performance with specimens other than those specified under *Specimen Collection and Storage* has not been evaluated.

   Laboratories may validate other collection devices (33, 36).

Gynecologic samples collected for preparation using the ThinPrep 2000 System or ThinPrep 5000 System should be collected using broom-type or endocervical brush/ plastic spatula combination collection devices.

K. 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.

L. The PreservCyt Solution has been validated as an alternative medium for testing with Aptima Combo 2 assay. PreservCyt Solution liquid Pap specimens processed using the ThinPrep 3000 Processor or other instruments have not been evaluated to test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using Aptima Combo 2 assay.

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

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

O. 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.

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

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

R. 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 Combo 2 assay should be processed using only the Aptima Specimen Transfer Kit.

S. 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

T. The performance of the Aptima Combo 2 assay has not been evaluated in adolescents less than 14 years of age.

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

V. **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.

	**Aptima Oil Reagent** ***Polydimethylsiloxane 100%*** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
	**Selection Reagent** Boric Acid 1-5% Sodium Hydroxide <1% **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
	**Target Capture Reagent** ***EDTA 1-5%*** H411 - Toxic to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/ face protection

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.*

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

Aptima Combo 2 Amplification Reagent

Aptima Combo 2 Enzyme Reagent

Aptima Combo 2 Probe Reagent

Aptima Combo 2 Target Capture Reagent B

APTIMA Positive Control, CT / Negative Control, GC

APTIMA Positive Control, GC / Negative Control, CT

B. The following reagents are stable when stored at 2°C to 30°C:

Aptima Combo 2 Amplification Reconstitution Solution

Aptima Combo 2 Enzyme Reconstitution Solution

Aptima Combo 2 Probe Reconstitution Solution

Aptima Combo 2 Selection Reagent

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Target Capture Reagent

Aptima Wash Solution

Aptima Buffer for Deactivation Fluid

Aptima Oil Reagent

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

E. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

F. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date passes, whichever comes first.

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

H. Reagents stored on-board the Tigris DTS system have 48 hours of on-board stability.

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

J. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Probe Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Probe Reagent should be limited accordingly.

K. 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).

L. Do not freeze the reagents.

Specimen Collection and Storage

The Aptima Combo 2 Assay is designed to detect the presence of CT and GC in the following specimens: clinician-collected endocervical, PreservCyt Solution liquid Pap specimens, vaginal, male urethral, and both male and female throat and rectal swab specimens; patient-collected vaginal, and both male and female throat and rectal swab specimens, and female and male urine specimens. Performance with specimens other than those collected with the following specimen collection kits has not been evaluated:

- Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- Aptima Urine Collection Kit for Male and Female Urine Specimens
- Aptima Multitest Swab Specimen Collection Kit
- 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. 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 Combo 2 Assay within 60 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after collection (see *Specimen Stability Studies*).
2. Urine specimens:
   a. Urine samples that are still in the primary collection container must be transported to the lab at 2°C to 30°C. Transfer the urine sample into the Aptima urine specimen transport tube within 24 hours of collection. Store at 2°C to 30°C and test within 30 days of collection.

b. After collection, transport the processed urine specimens in the Aptima urine specimen transport tube at 2°C to 30°C and store at 2°C to 30°C until tested. Processed urine specimens should be assayed with the Aptima Combo 2 Assay within 30 days of collection. If longer storage is needed, freeze at -20°C to -70°C 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 and/or GC 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 2000, ThinPrep 3000, or ThinPrep 5000 Processor Operator's Manual—Addendum* 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 2000 processor, process the PreservCyt Solution liquid Pap specimen in accordance with the *ThinPrep 2000 Processor Operator's Manual* and the Aptima Specimen Transfer Kit package insert. If testing the specimen after using the ThinPrep 5000 processor, process the PreservCyt Solution liquid Pap specimen in accordance with the ThinPrep 5000 Processor Operator's Manual and the Aptima Specimen Transfer Kit package insert. Transfer 1 mL of the fluid remaining in 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 Combo 2 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 at -20°C to -70°C for 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 penetrable cap and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination**.

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

Tigris DTS System

Reagents for the Aptima Combo 2 assay for CT and GC are listed below for the Tigris DTS system. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima Combo 2 Assay Kit, 250 tests (2 boxes and 1 Controls kit) (Cat. No. PRD-05572 and PRD-05572B)

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

Symbol	Component	Quantity
A	**Aptima Combo 2 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Aptima Combo 2 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Aptima Combo 2 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
TCR-B	**Aptima Combo 2 Target Capture Reagent B** *Non-infectious nucleic acids in a buffered solution containing < 5% detergent.*	1 x 0.61 mL

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

Symbol	Component	Quantity
AR	**Aptima Combo 2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima Combo 2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PR	**Aptima Combo 2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL
S	**Aptima Combo 2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL
TCR	**Aptima Combo 2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 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	**Aptima 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	**Aptima 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 conductive, liquid sensing		10612513 (Tecan)
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 for Male and Female Urine Specimens		105575
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution		—
Water for the Tigris DTS System *consult the Tigris DTS System Operator's Manual for specifications*		—

		Cat. No.
Disposable gloves		—
SysCheck calibration standard		301078
Aptima penetrable caps		105668
Replacement non-penetrable caps		103036A
Replacement caps for the 250-test kits		—
Amplification and Probe reagent reconstitution solutions	*CL0041 (100 caps)*	
Enzyme Reagent reconstitution solution	*501616 (100 caps)*	
TCR and Selection reagent	*CL0040 (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 vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, 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 bottle opening (Figure 1, Step 2).

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

g. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 1, 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 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.

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

j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

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

Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

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

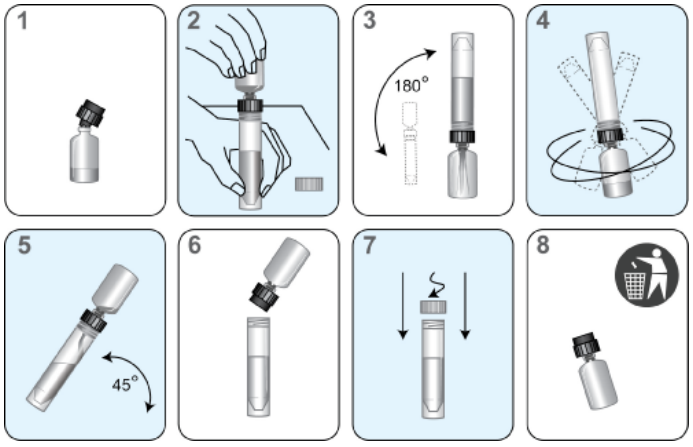

Figure 1. Tigris DTS System or Panther System Reconstitution Process

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

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

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.
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, CT / Negative Control, GC must be in the first position and second to last position of a worklist. This control label is pink. The label text is “CONTROL + CT PCT / CONTROL – GC NGC”. The Positive Control, GC / Negative Control, CT must be in the second position and last position of a worklist. This control label is blue-green. The label text is “CONTROL + GC PGC / CONTROL – CT NCT”.
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 swab transport medium, 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 or GC positive or equivocal, see *Test Interpretation — QC Patient Results*. For additional contamination monitoring information specific to the Tigris DTS system, see the *Tigris DTS System Operator’s Manual*.

Panther System

Reagents for the Aptima Combo 2 assay for CT and GC are listed below for the Panther system. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima Combo 2 Assay Kit

100 tests (2 boxes and 1 Controls kit) (Cat. No. PRD-05576)

250 tests (2 boxes and 1 Controls kit) (Cat. No. PRD-05571)

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

Symbol	Component	Quantity 250 test kit	Quantity 100 test kit
A	**Aptima Combo 2 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial	1 vial
E	**Aptima Combo 2 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial	1 vial
P	**Aptima Combo 2 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial	1 vial
TCR-B	**Aptima Combo Target Capture Reagent B** *Non-infectious nucleic acids in a buffered solution containing < 5% detergent.*	1 x 0.61 mL	1 x 0.30 mL

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

Symbol	Component	Quantity 250 test kit	Quantity 100 test kit
AR	**Aptima Combo 2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL	1 x 11.9 mL
ER	**Aptima Combo 2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL	1 x 6.3 mL
PR	**Aptima Combo 2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL	1 x 15.2 mL
S	**Aptima Combo 2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL	1 x 43.0 mL

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

Symbol	Component	Quantity 250 test kit	Quantity 100 test kit
TCR	**Aptima Combo 2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 mL	1 x 26.0 mL
	Reconstitution Collars	3	3
	Master Lot Barcode Sheet	1 sheet	1 sheet

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

Symbol	Component	Quantity
PCT/NGC	**Aptima 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	**Aptima 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 conductive, liquid sensing	10612513 (Tecan)
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 for Male and Female Urine Specimens	105575
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable gloves	—
SysCheck calibration standard	301078
Aptima penetrable caps	105668
Replacement non-penetrable caps	103036A
Replacement caps for the 250-test kits	—
Amplification and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
Enzyme Reagent reconstitution solution	*501616 (100 caps)*
TCR and Selection reagent	*CL0040 (100 caps)*
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

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 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 vial and firmly insert the notched end of the reconstitution collar into the 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 bottle opening (Figure 2, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 2, Step 3).
   g. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (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 plastic bottle.
   i. Remove the reconstitution collar and glass vial (Figure 2, Step 6).
   j. Recap the plastic bottle. Record operator initials and the reconstitution date on the label (Figure 2, Step 7).
   k. Discard the reconstitution collar and glass vial (Figure 2, Step 8).

   Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

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

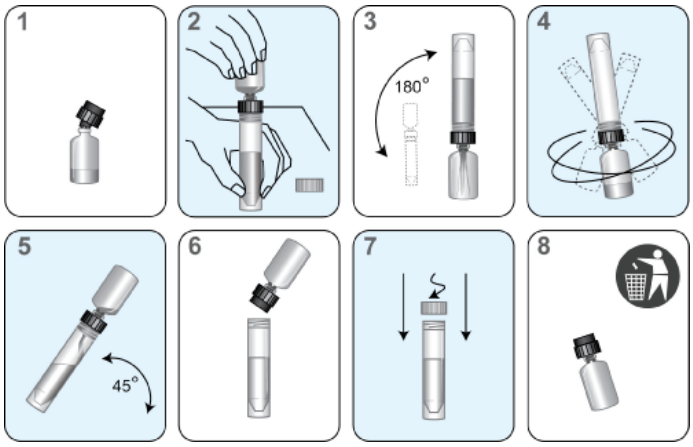

Figure 2. Tigris DTS System or Panther System Reconstitution Process

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

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

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

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 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 Panther Aptima assay software, 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 kit up to 24 hours **unless:**
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

B. Temperature

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

C. Glove Powder

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

D. Lab Contamination Monitoring Protocol for the Panther system

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring

frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

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

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 swab transport medium, 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 or GC 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 Aptima Combo 2 protocol, and presented as individual CT and GC test results. A test result may be a negative, equivocal, positive, or invalid as determined by the kinetic type and total RLU in the detection step (see below). A test result may be invalid due to a parameter outside the normal expected ranges. Initial equivocal and invalid test results should be retested.

Kinetic Type	Total RLU (x1000) to give CT Result		
	Negative	Equivocal	Positive
CT only	1 to < 25	25 to < 100	100 to < 4,500
CT and GC	1 to < 85	85 to < 250	250 to < 4,500
CT indeterminate	1 to < 85	85 to < 4,500	N/A

Kinetic Type	Total RLU (x1000) to give GC Result		
	Negative	Equivocal	Positive
GC only	1 to < 60	60 to < 150	150 to < 4,500
GC and CT	1 to < 85	85 to < 250	250 to < 4,500
GC indeterminate	1 to < 85	85 to < 4,500	N/A

B. Quality Control Results and Acceptability

The Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control, CT 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 Positive Control, CT / Negative Control, GC serves as the negative control for the GC test results. The Positive Control, GC / Negative Control, CT serves as the negative control for the CT test results. If desired, a dual negative control furnished by the user can be added to monitor assay background. 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 PreservCyt Solution liquid Pap specimens.

The Positive Controls must produce the following test results:

Control	Total RLU (x1000)	CT Result	GC Result
Positive Control, CT/ Negative Control, GC	≥ 100 and < 3,000	Positive	Negative
Positive Control, GC/ Negative Control, CT	≥ 150 and < 3,000	Negative	Positive

1. The Aptima assay software automatically evaluates the controls according to the above criteria and will report the Run Status as PASS if the run control criteria are met, and FAIL if the run control criteria are not met.
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 (section 493.1256).
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 Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control, CT provided in the kit 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 in appropriate transport media (PreservCyt Solution, STM) 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, PreservCyt Solution liquid Pap, and urine specimen results. (See Notes below.)
   a. Initial results

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

Notes:

- Careful consideration of performance data is recommended for interpreting Aptima Combo 2 assay results for asymptomatic individuals or any individuals in low prevalence populations.
- The first valid result for each analyte is the result that should be reported.
- A negative result does not preclude the presence of a CT or GC 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, or specimen mix-up.
- As is true for all non-culture methods, a positive specimen obtained from a patient after therapeutic treatment cannot be interpreted as indicating the presence of viable CT or GC.
- As is true for all urine test methods, a negative urine result for a female patient who is clinically suspected of having a chlamydial or gonococcal infection does not rule out the presence of CT or GC in the urogenital tract.
- Vaginal swab is recommended for female patients who are clinically suspected of having a chlamydial or gonococcal infection (29, 40).
- 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. Swab specimens were evaluated in the Aptima Combo 2 Assay on the DTS system for interference by blood, gynecological lubricants, and spermicides. Urine specimens were evaluated for interference by blood, commonly used vitamins, minerals, and over-the-counter pain relievers. Blood interference was evaluated on the Tigris DTS system and Panther system. Swab specimens were also evaluated on the Panther system for interferences by cold sore medication, lip balms, cough suppressants, toothpaste, mouthwash, hemorrhoidal cream, laxative, anti-diarrheal medications, antacids, and feces. The data indicated no assay interference by these substances.

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

D. The presence of mucus in endocervical specimens does not interfere with the detection of CT or GC by the Aptima Combo 2 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.

E. This assay has been tested using only the following specimens:

- Clinician-collected endocervical, vaginal, male urethral, throat, and rectal swab specimens
- Clinician-collected PreservCyt Solution liquid Pap specimens
- Patient-collected vaginal, throat, and rectal swab specimens
- Patient-collected female and male urine specimens

Performance with specimens other than those collected with the following specimen collection kits has not been evaluated:

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

F. 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.

G. The Aptima Combo 2 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, the CDC recommends retesting (8).

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

I. Therapeutic failure or success cannot be determined with the Aptima Combo 2 Assay since nucleic acid may persist following appropriate antimicrobial therapy.

J. Results from the Aptima Combo 2 assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

K. 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.

L. The Aptima Combo 2 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.

M. For the vaginal swab, endocervical swab, male urethral swab and urine specimen clinical studies, performance for detecting CT and GC 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.

N. For the PreservCyt Solution liquid Pap specimen clinical studies, the Aptima Combo 2 assay performance for detecting CT and GC 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.

O. 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.

P. PreservCyt Solution liquid Pap specimens processed with instruments other than the ThinPrep 2000 or ThinPrep 5000 processors have not been evaluated for use in Aptima assays.

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

R. The patient-collected vaginal, throat, and rectal swab specimen applications are limited to health care facilities where support/counseling is available to explain procedures and precautions.

S. The Aptima Combo 2 assay has not been validated for use with specimens collected by patients at home.

T. The performance of the Aptima Combo 2 assay has not been evaluated in adolescents less than 14 years of age.

U. 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.

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

W. There is no evidence of degradation of nucleic acids in PreservCyt Solution. If a PreservCyt Solution liquid Pap specimen has small numbers of CT and GC cellular material, uneven distribution of this cellular material may occur. Also, when compared to

direct sampling with the Aptima Swab Transport Media, 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.

X. Customers must independently validate an LIS transfer process.

Aptima Combo 2 Expected Values

Note: *The following results were generated with the original version of the Aptima Combo 2 assay using the DTS systems.*

Prevalence

The prevalence of CT and/or GC disease 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 three CT and GC disease outcomes as determined by the Aptima Combo 2 Assay is shown in Tables 1a, 1b, and 1c for three multi-center clinical studies by clinical site and overall.

Prevalence of *C. trachomatis* and/or *N. gonorrhoeae* Disease as Determined by the Aptima Combo 2 Assay Results by Clinical Site

Table 1a: Endocervical and Male Urethral Swab and Urine Specimens

Site	Endocervical and Male Urethral Swab % Prevalence (# positive/# tested)						Urine % Prevalence (# positive/# tested)					
	CT+/GC+		CT+/GC-		CT-/GC+		CT+/GC+		CT+/GC-		CT-/GC+	
1	10.0	(39/392)	12.8	(50/392)	14.5	(57/392)	8.4	(33/395)	12.9	(51/395)	13.9	(55/395)
2	7.0	(13/186)	12.9	(24/186)	6.5	(12/186)	5.3	(13/245)	13.9	(34/245)	8.6	(21/245)
3	10.4	(48/462)	22.9	(106/462)	14.3	(66/462)	10.3	(48/465)	20.9	(97/465)	12.7	(59/465)
4	3.3	(9/270)	12.2	(33/270)	7.0	(19/270)	3.3	(9/270)	11.5	(31/270)	6.7	(18/270)
5	1.9	(10/533)	8.4	(45/533)	2.3	(12/533)	2.1	(12/567)	9.4	(53/567)	1.8	(10/567)
6	6.3	(43/678)	12.8	(87/678)	16.2	(110/678)	5.9	(40/681)	10.9	(74/681)	13.5	(92/681)
7	4.4	(11/252)	8.7	(22/252)	21.8	(55/252)	4.1	(12/295)	9.2	(27/295)	18.0	(53/295)
All	6.2	(173/2773)	13.2	(367/2773)	11.9	(331/2773)	5.7	(167/2918)	12.6	(367/2918)	10.6	(308/2918)

Table 1b: Patient-Collected Vaginal Swab and Clinician-Collected Vaginal Swab Specimens

Site	Patient-Collected Vaginal Swab % Prevalence (# positive / # tested)						Clinician-Collected Vaginal Swab % Prevalence (# positive / # tested)					
	CT+/GC+		CT+/GC-		CT-/GC+		CT+/GC+		CT+/GC-		CT-/GC+	
1	1.8	(4/220)	16.4	(36/220)	4.1	(9/220)	3	(7/230)	15.7	(36/230)	3.5	(8/230)
2	9.6	(19/198)	18.7	(37/198)	6.6	(13/198)	9.5	(19/199)	18.1	(36/199)	7	(14/199)
3	0.9	(1/111)	9	(10/111)	2.7	(3/111)	0.9	(1/113)	9.7	(11/113)	1.8	(2/113)
4	0.4	(1/266)	9	(24/266)	1.9	(5/266)	0.4	(1/267)	11.2	(30/267)	2.2	(6/267)
5	0.5	(1/199)	7.5	(15/199)	0.5	(1/199)	0.5	(1/199)	7	(14/199)	0.5	(1/199)
6	2.8	(8/290)	10	(29/290)	5.5	(16/290)	2	(6/296)	12.2	(36/296)	5.4	(16/296)
7	0	(0/102)	11.8	(12/102)	0	(0/102)	0	(0/102)	9.8	(10/102)	0	(0/102)
8	0	(0/48)	8.3	(4/48)	2.1	(1/48)	0	(0/51)	7.8	(4/51)	2	(1/51)
All	2.4	(34/1434)	11.6	(167/1434)	3.3	(48/1434)	2.4	(35/1457)	12.1	(177/1457)	3.3	(48/1457)

Table 1c: PreservCyt Solution Liquid Pap Specimen

Site	PreservCyt Solution liquid Pap % Prevalence (# positive/# tested)		
	CT+/GC+	CT+/GC-	CT-/GC+
1	3.0 (3/100)	13.0 (13/100)	2.0 (2/100)
2	0 (0/124)	3.2 (4/124)	0.8 (1/124)
3	0.4 (2/475)	6.1 (29/475)	0.4 (2/475)
4	0.4 (1/287)	4.2 (12/287)	0 (0/287)
5	0 (0/297)	5.1 (15/297)	1.0 (3/297)
6	0 (0/364)	5.5 (20/364)	0.6 (2/364)
ALL	0.4 (6/1647)	5.6 (93/1647)	0.6 (10/1647)

The CT and GC prevalence were calculated using the Aptima Combo 2 assay results of PreservCyt Solution liquid Pap specimen.

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

The estimated positive and negative predictive values (PPV and NPV) for different prevalence rates using the Aptima Combo 2 Assay are shown in Tables 2 and 3 for CT and GC, respectively. These calculations are based on a hypothetical prevalence and the overall sensitivity and specificity calculated from the patient infected status for two multi-center clinical studies. The overall sensitivity and specificity for CT was 96.1% and 98.0%, respectively (Table 2). The overall sensitivity and specificity for GC was 97.8% and 99.2%, respectively (Table 3). The actual PPV and NPV calculated using the clinical trial data are shown in Tables 6a and 10a (swab and urine specimens), Tables 6b and 10b (vaginal swab specimens), and Tables 6c and 10c (PreservCyt Solution liquid Pap specimens).

Table 2: Hypothetical PPV and NPV for CT

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	96.1	98.0	33.1	100.0
2	96.1	98.0	50.0	99.9
5	96.1	98.0	72.0	99.8
10	96.1	98.0	84.5	99.6
15	96.1	98.0	89.6	99.3
20	96.1	98.0	92.4	99.0
25	96.1	98.0	94.2	98.7
30	96.1	98.0	95.4	98.3

Table 3: Hypothetical PPV and NPV for GC

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	97.8	99.2	55.3	100.0
2	97.8	99.2	71.4	100.0
5	97.8	99.2	86.6	99.9
10	97.8	99.2	93.2	99.7
15	97.8	99.2	95.6	99.6
20	97.8	99.2	96.8	99.4
25	97.8	99.2	97.6	99.2
30	97.8	99.2	98.1	99.0

Aptima Combo 2 Clinical Performance

Note: *The following results were generated with the original version of the Aptima Combo 2 assay using the DTS systems.*

See *Tigris DTS System Clinical Specimen Agreement* following the *Aptima Combo 2 Analytical Performance* section for Tigris DTS system-specific clinical performance.

Clinical Study Results

Performance for the Aptima Combo 2 assay on DTS systems was established in three multi-center clinical studies, conducted in North America. The first multi-center clinical study evaluated clinician-collected endocervical and male urethral swabs and male and female urine specimens from 1,363 male and 1,569 female subjects enrolled at seven geographically diverse clinical sites. The second multi-center clinical study evaluated patient-collected and clinician-collected vaginal swab specimens from 1,464 female subjects enrolled at eight geographically diverse clinical sites. The third multi-center clinical study evaluated PreservCyt Solution liquid Pap specimens from 1,647 subjects enrolled at six clinical sites. In performance calculations based on symptom status, 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.

Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study

In the endocervical swab, urethral swab, and urine specimen multi-center clinical study, 2,932 symptomatic and asymptomatic male and female subjects attending STD, OB/GYN and family planning clinics were enrolled in the study. As many as three urethral swabs and a urine specimen were collected from male subjects and four endocervical swabs and a urine specimen were collected from female subjects. For males providing one urethral swab, testing included GC culture only. For males providing three swabs, testing included GC culture, the Aptima Combo 2 assay, and a commercially-available NAAT for CT and GC. Testing on endocervical swabs included the Aptima Combo 2 assay, two commercially-available NAATs for CT, one commercially-available NAAT for GC, and GC culture. The GC culture swab was collected first and the collection order for the remaining swabs was rotated to minimize collection bias. Urine was tested by the Aptima Combo 2 assay, two commercially-available NAATs for CT, and one commercially-available amplified assay for GC. The commercially-available amplification assays were used as reference assays in this Aptima Combo 2 assay clinical study.

All performance calculations were based on the total number of Aptima Combo 2 assay endocervical and male urethral swab and male and female urine specimens compared to a patient infected status algorithm for each gender. In each gender-specific algorithm, the designation of a subject as being infected, not infected, or inconclusive was based on the combined results of the reference NAAT endocervical and male urethral swab and urine results. For CT infected status, any two positive reference NAAT results by any combination of swab and urine designated the subject as infected. If all reference assay results were negative, the subject was designated not infected. If there was one positive result only, the subject was designated inconclusive. For GC infected status, a positive culture, or positive swab and urine results by the amplified reference assay, designated the subject as infected. A negative culture and a single positive result by the amplified reference assay resulted in an inconclusive status. If all reference assay results were negative, the subject was designated not infected. Tables 7a, 7b, 7c, 8, 11a, 11b, 11c, and 12 summarize the frequency of test outcomes for the two reference NAATs and Aptima Combo 2 assay for clinical study subjects.

Aptima Combo 2 assay results from the clinician-collected endocervical and male urethral swab, and male and female urine specimens were compared to the patient infected status algorithm for determination of sensitivity, specificity, and predictive values. A total of 15,661 CT and 14,144 GC test results were used in the data analysis. Sensitivity and specificity for CT by gender, specimen type, and symptom status are presented in Table 5a. Table 6a shows the Aptima Combo 2 assay sensitivity, specificity, and predictive values for CT compared to patient infected status for each clinical site and overall. Sensitivity and specificity for detection of GC by gender, specimen type and symptom status are presented in Table 9a. Table 10a shows the GC sensitivity, specificity, and predictive values for the Aptima Combo 2 assay compared to patient infected status for each clinical site and overall. Samples that were Aptima Combo 2 assay positive and infected patient status negative (i.e., apparent false positives) were tested in Hologic alternate amplification assays for CT and GC. These assays amplify CT and GC sequences which are different from those amplified in the Aptima Combo 2 assay. Testing was done on a per specimen basis (i.e., not necessarily on paired swab and urine specimens) and the results of the alternate amplification assays were not used to change the original patient categorizations (Tables 5a and 9a).

Endocervical swab specimens were evaluated for the impact of blood on CT and GC assay performance. Of the 2,454 specimens evaluated for CT performance, 234 (9.5%) were bloody. Of the 2,829 specimens evaluated for GC performance, 247 (8.7%) were bloody. Neither the CT nor GC assay performance was statistically different for bloody specimens as compared to non-bloody specimens. Additional data on blood testing can be found in *Interfering Substances*.

Performance of the assay with endocervical swab and urine specimens from pregnant females was assessed in the clinical study. For CT, sensitivity for endocervical swab and urine specimens was 100% (8/8) and 100% (8/8), respectively. Specificity for endocervical swab and urine specimens was 95.8% (23/24) and 100% (24/24), respectively. For GC, sensitivity for endocervical swab and urine specimens was 100% (8/8) and 100% (8/8), respectively. Specificity for endocervical swab and urine specimens was 100% (26/26) and 100% (26/26), respectively.

Of the 11,406 Aptima Combo 2 assay test results from this multi-center clinical study, three CT results and nine GC results were equivocal on repeat testing and were excluded from the analysis. One specimen was invalid for both CT and GC results and was excluded from the study.

Vaginal Swab Specimen Clinical Study

In the vaginal swab multi-center clinical study, 1,464 symptomatic and asymptomatic female subjects attending STD, OB/GYN, teen, and family planning clinics were enrolled into the clinical study. Of the 646 asymptomatic subjects enrolled in the study, two were less than 16 years of age, 158 were between the ages of 16 and 20, 231 were between the ages of 21 and 25, and 255 were more than 25 years of age. Of the 818 symptomatic subjects enrolled in the study, 160 were between the ages of 16 and 20, 324 were between the ages of 21 and 25, and 334 were more than 25 years of age. Five specimens were collected from each eligible subject; one urine specimen, one patient-collected vaginal swab, one clinician-collected vaginal swab, and two randomized endocervical swabs. Aptima Combo 2 assay results were generated from the two vaginal swabs, one of the endocervical swabs, and an aliquot of the urine specimen. The second endocervical swab and a second aliquot of the urine specimen were tested using another commercially-available NAAT for CT and another commercially-available NAAT for GC. Endocervical swab and urine specimens tested in the Aptima Combo 2 assay and the other commercially-available NAATs were used as reference NAATs to determine infected status for each subject in the vaginal swab specimen clinical study. 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 Combo 2 assay patient-collected and clinician-collected vaginal swab results compared to a patient infected status algorithm. A total of 2,073 CT and 2,073 GC vaginal swab test results were used in the data analysis. In the algorithm, the designation of a subject as being infected or not infected with CT or GC 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 or GC 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. Tables 7b and 11b summarize the number of results from symptomatic and asymptomatic subjects designated as infected or non-infected with CT or GC, respectively, according to the patient infected status algorithm. For this clinical study, two commercially-available NAATs were used to determine GC-infected status. Culture was not used as a reference test since the Aptima Combo 2 assay has already been evaluated against culture for other specimen types (refer to the *Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study* for details).

Sensitivity and specificity for CT by gender, specimen type and symptom status are presented in Table 5b. Table 6b shows the Aptima Combo 2 assay sensitivity, specificity, and predictive values for CT compared to patient infected status for each clinical site and overall. Sensitivity and specificity for detection of GC by gender, specimen type and symptom status are presented in Table 9b. Table 9b shows the GC sensitivity, specificity, and predictive values for the Aptima Combo 2 assay compared to patient infected status for each clinical site and overall. Samples that were Aptima Combo 2 assay positive and infected patient status negative (i.e., apparent false positives) were tested in alternate TMA assays for CT and GC; these alternate TMA assays target sequences which are unique from those targeted in the Aptima Combo 2 assay. The results of the alternate TMA assays were not used to change the original patient categorizations (Tables 5b and 9b).

Of the 1,464 subjects enrolled, there were 13 subjects with unknown CT patient infected status and 14 subjects with unknown GC 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 5,782 Aptima Combo 2 assay vaginal swab results from the multi-center clinical study, there was a small percentage (28, 0.5%) of vaginal swab specimens that initially tested invalid or equivocal for CT or GC. Upon repeat testing only three CT results and two GC results were equivocal and were excluded from the analysis. No specimens tested invalid on repeat testing.

PreservCyt Solution Liquid Pap Specimen Clinical Study

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 and GC. 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 available subjects, 1,288 were asymptomatic subjects and 359 were symptomatic subjects. Subjects were enrolled from sites with CT prevalence that ranged from 3.2% to 14.0% and GC prevalence that ranged from 0% to 5.0%. Two specimens were collected from each eligible subject: one PreservCyt Solution liquid Pap specimen and one endocervical swab. 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 Combo 2 assay. The PreservCyt Solution liquid Pap specimens and endocervical swab specimens were tested with the Aptima Combo 2 assay.

Sensitivity and specificity for PreservCyt Solution liquid Pap specimens were calculated by comparing results to a patient infected status algorithm. In the algorithm, the designation of a subject as being infected or non-infected with CT or GC was based on endocervical swab specimen results from two commercially-available NAATs (Tables 7c and 11c). For CT, the reference NAATs included the Aptima Combo 2 assay and the Aptima CT assay. For GC, the reference NAATs included the Aptima Combo 2 assay and the Aptima GC assay. Positive results from both reference NAATs were required to establish an *infected* patient. A *non-infected* patient was established if the results from the two reference NAATs disagreed or were negative.

Sensitivity and specificity for CT in PreservCyt Solution liquid Pap specimens tested in the Aptima Combo 2 assay, by symptom status and overall, is presented in Table 5c. For CT, overall sensitivity was 96.7% (87/90). In symptomatic and asymptomatic subjects, sensitivity was 96.7% (29/30) and 96.7% (58/60), respectively. Overall specificity for CT PreservCyt Solution liquid Pap specimens was 99.2% (1545/1557). In symptomatic and asymptomatic subjects, specificity was 98.5% (324/329) and 99.4% (1221/1228), respectively. Table 6c shows the Aptima Combo 2 assay sensitivity and specificity values for CT in PreservCyt Solution liquid Pap specimens by clinical site and overall. For CT, the sensitivity ranged from 92.9% to 100%. The specificity ranged from 97.7% to 100%.

Sensitivity and specificity for GC in PreservCyt Solution liquid Pap specimens tested in the Aptima Combo 2 assay, by symptom status and overall, is presented in Table 9c. For GC, overall sensitivity was 92.3% (12/13). In symptomatic and asymptomatic subjects, sensitivity was 100% (7/7) and 83.3% (5/6), respectively. Overall specificity for GC PreservCyt Solution liquid Pap specimens was 99.8% (1630/1634). In symptomatic and asymptomatic subjects, specificity was 100% (352/352) and 99.7% (1278/1282), respectively. Table 10c shows the Aptima Combo 2 assay sensitivity and specificity values for GC in PreservCyt Solution liquid Pap specimens by clinical site and overall. For GC, the sensitivity ranged from 80.0% to 100%. Specificity ranged from 99.0% to 100%.

The distribution of cervical sampling devices used in this clinical study according to clinical site is summarized in Table 4.

Table 4: Summary of Cervical Sampling Devices Used in the PreservCyt Solution Liquid Pap Specimen Study

Cervical sampling device	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

Chlamydia trachomatis Performance Tables

C. trachomatis Sensitivity and Specificity

Table 5a: Aptima Combo 2 Assay Specimens vs. Patient Infected Status

Specimen		Symptoms Status	N	TP	FP[4]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Male	Swab	Sympt	676	190	15[a]	464	7	96.4% (92.8–98.6)	96.9% (94.9–98.2)
		Asympt	388	70	5[b]	309	4	94.6% (86.7–98.5)	98.4% (96.3–99.5)
		All[1]	1065	260	20[c]	774	11	95.9% (92.9–98.0)	97.5% (96.1–98.5)
	Urine	Sympt	694	199	8[d]	484	3	98.5% (95.7–99.7)	98.4% (96.8–99.3)
		Asympt	400	77	4[e]	316	3	96.3% (89.4–99.2)	98.8% (96.8–99.7)
		All[1]	1095	276	12[f]	801	6	97.9% (95.4–99.2)	98.5% (97.4–99.2)
Female	Swab	Sympt	819	133	22[g]	653	11	92.4% (86.7–96.1)	96.7% (95.1–97.9)
		Asympt	569	61	6[h]	501	1	98.4% (91.3–100)	98.8% (97.4–99.6)
		All[2]	1389	195	28[i]	1154	12	94.2% (90.1–97.0)	97.6% (96.6–98.4)
	Urine	Sympt	821	136	8[j]	668	9	93.8% (88.5–97.1)	98.8% (97.7–99.5)
		Asympt	569	60	5[k]	502	2	96.8% (88.8–99.6)	99.0% (97.7–99.7)
		All[2]	1391	197	13[l]	1170	11	94.7% (90.7–97.3)	98.9% (98.1–99.4)
Total	Swab	Sympt	1495	323	37[m]	1117	18	94.7% (91.8–96.8)	96.8% (95.6–97.7)
		Asympt	957	131	11[n]	810	5	96.3% (91.6–98.8)	98.7% (97.6–99.3)
		All[3]	2454	455	48[o]	1928	23	95.2% (92.9–96.9)	97.6% (96.8–98.2)
	Urine	Sympt	1515	335	16[p]	1152	12	96.5% (94.0–98.2)	98.6% (97.8–99.2)
		Asympt	969	137	9[q]	818	5	96.5% (92.0–98.8)	98.9% (97.9–99.5)
		All[3]	2486	473	25[r]	1971	17	96.5% (94.5–98.0)	98.7% (98.2–99.2)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

[1] Includes 1 male subject for whom symptoms were not reported.

[2] Includes 1 female subject for whom symptoms were not reported.

[3] Includes 1 male and 1 female subject for whom symptoms were not reported.

[4] CT Alternate TMA results represent # positive results/# specimens tested: a: 11/14; b: 3/5; c: 14/19; d: 4/8; e: 0/4; f: 4/12; g: 18/22; h: 4/6; i: 22/28; j: 2/8; k: 1/5; l: 3/13, m: 29/36, n: 7/11, o: 36/47, p: 6/16, q: 1/9, and r: 7/25.

Table 5b: Aptima Combo 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Symptom Status	N	TP	FP[1]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Patient-Collected	Vaginal Swab	Asympt	628	60	18[a]	549	1	98.4% (91.2–100)	96.8% (95.0–98.1)
Clinician-Collected	Vaginal Swab	Sympt	809	111	25[b]	669	4	96.5% (91.3–99.0)	96.4% (94.7–97.7)
		Asympt	636	59	16[c]	559	2	96.7% (88.7–99.6)	97.2% (95.5–98.4)
		All	1445	170	41[d]	1228	6	96.6% (92.7–98.7)	96.8% (95.6–97.7)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

[1] CT TMA Alternate Amplification results represent # positive results/# specimens tested: a: 15/18, b: 17/25, c: 15/16, and d: 32/41.

Table 5c: Aptima Combo 2 Assay PreservCyt Specimens vs. Patient Infected Status

Symptom Status	AC2/CT PreservCyt Result	+/+	+/-	-/+	-/-	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Asympt	Positive	58	1	0	6	96.7% (88.5 - 99.6)	99.4% (98.8 - 99.8)
	Negative	2	1	12	1208		
	Total	60	2	12	1214		
Sympt	Positive	29	0	0	5	96.7% (82.8 - 99.9)	98.5% (96.5 - 99.5)
	Negative	1	3	4	317		
	Total	30	3	4	322		
All	Positive	87	1	0	11	96.7% (90.6 - 99.3)	99.2% (98.7 - 99.6)
	Negative	3	4	16	1525		
	Total	90	5	16	1536		

+/+ = Positive Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the ACT assay.

+/- = Positive Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the ACT assay.

-/+ = Negative Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the ACT assay.

-/- = Negative Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the ACT assay.

C. trachomatis Performance by Clinical Site

Table 6a: Aptima Combo 2 Assay Specimen vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Male	Swab	1	157	35	6	115	1	22.9	97.2% (85.5–99.9)	95.0% (89.5–98.2)	85.4	99.1
		2	93	19	2	72	0	20.4	100% (82.4–100)	97.3% (90.6–99.7)	90.5	100
		3	248	76	5	165	2	31.5	97.4% (91.0–99.7)	97.1% (93.3–99.0)	93.8	98.8
		4	51	12	1	38	0	23.5	100% (73.5–100)	97.4% (86.5–99.9)	92.3	100
		5	138	24	0	113	1	18.1	96.0% (79.6–99.9)	100% (96.8–100)	100	99.1
		6	353	74	6	268	5	22.4	93.7% (85.8–97.9)	97.8% (95.3–99.2)	92.5	98.2
		7	25	20	0	3	2	88.0*	90.9% (70.8–98.9)	100% (29.2–100)	100	60.0
		ALL	1065	260	20	774	11	25.4	95.9% (92.9–98.0)	97.5% (96.1–98.5)	92.9	98.6
	Urine	1	157	35	6	115	1	22.9	97.2% (85.5–99.9)	95.0% (89.5–98.2)	85.4	99.1
		2	96	22	1	73	0	22.9	100% (84.6–100)	98.6% (92.7–100)	95.7	100
		3	249	78	2	169	0	31.3	100% (95.4–100)	100% (95.8–99.9)	97.5	100
		4	51	12	0	39	0	23.5	100% (73.5–100)	98.8% (91.0–100)	100	100
		5	162	31	2	129	0	19.1	100% (88.8–100)	98.5% (94.6–99.8)	93.9	100
		6	353	74	1	273	5	22.4	93.7% (85.8–97.9)	99.6% (98.0–100)	98.7	98.2
		7	27	24	0	3	0	88.9*	100% (85.8–100)	100% (29.2–100)	100	100
		ALL	1095	276	12	801	6	25.8	97.9% (95.4–99.2)	98.5% (97.4–99.2)	95.8	99.3
Female	Swab	1	150	34	4	110	2	24.0	94.4% (81.3–99.3)	96.5% (91.3–99.0)	89.5	98.2
		2	81	11	1	68	1	14.8	91.7% (61.5–99.8)	98.6% (92.2–100)	91.7	98.6
		3	184	51	13	114	6	31.0	89.5% (78.5–96.0)	89.8% (83.1–94.4)	79.7	95.0
		4	196	27	2	167	0	13.8	100% (87.2–100)	98.8% (95.8–99.9)	93.1	100
		5	370	27	1	341	1	7.6	96.4% (81.7–99.9)	99.7% (98.4–100)	96.4	99.7
		6	274	35	7	230	2	13.5	94.6% (81.8–99.3)	97.0% (94.0–98.8)	83.3	99.1
		7	134	10	0	124	0	7.5	100% (69.2–100)	100% (97.1–100)	100	100
		ALL	1389	195	28	1154	12	14.9	94.2% (90.1–97.0)	97.6% (96.6–98.4)	87.4	99.0
	Urine	1	150	34	4	110	2	24.0	94.4% (81.3–99.3)	96.5% (91.3–99.0)	89.5	98.2
		2	81	12	1	68	0	14.8	100% (73.5–100)	98.6% (92.2–100)	92.3	100
		3	185	54	3	125	3	30.8	94.7% (85.4–98.9)	97.7% (93.3–99.5)	94.7	97.7
		4	196	24	2	167	3	13.8	88.9% (70.8–97.6)	98.8% (95.8–99.9)	92.3	98.2
		5	369	28	2	338	1	7.9	96.6% (82.2–99.9)	99.4% (97.9–99.9)	93.3	99.7
		6	276	35	1	238	2	13.4	94.6% (81.8–99.3)	99.6% (97.7–100)	97.2	99.2
		7	134	10	0	124	0	7.5	100% (69.2–100)	100% (97.1–100)	100	100
		ALL	1391	197	13	1170	11	15.0	94.7% (90.7–97.3)	98.9% (98.1–99.4)	93.8	99.1

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

* Prevalence over-estimated due to initial collection being limited to screening for symptomatic subjects.

Table 6b: Aptima Combo 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev. (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Patient-Collected	Vaginal Swab	1	70	14	3	53	0	20.0	100% (76.8–100)	94.6% (85.1–98.9)	82.4	100
		2	45	13	3	29	0	28.9	100% (75.3–100)	90.6% (75.0–98.0)	81.3	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)	99.7% (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	2	65	0	10.7	100% (63.1–100)	97.0% (89.6–99.6)	80.0	100
		7	68	5	1	62	0	7.4	100% (47.8–100)	98.4% (91.5–100)	83.3	100
		8	43	3	1	39	0	7.0	100% (29.2–100)	97.5% (86.8–99.9)	75.0	100
		ALL	628	60	18	549	1	9.7	98.4% (91.2–100)	96.8% (95.0–98.1)	76.9	99.8
Clinician-Collected	Vaginal Swab	1	227	34	9	182	2	15.9	94.4% (81.3–99.3)	95.3% (91.2–97.8)	79.1	98.9
		2	196	50	5	139	2	26.5	96.2% (86.8–99.5)	96.5% (92.1–98.9)	90.9	98.6
		3	113	9	3	101	0	8.0	100% (66.4–100)	97.1% (91.8–99.4)	75.0	100
		4	262	19	11	231	1	7.6	95.0% (75.1–99.9)	95.5% (92.0–97.7)	63.3	99.6
		5	199	13	2	184	0	6.5	100% (75.3–100)	98.9% (96.2–99.9)	86.7	100
		6	296	33	9	254	0	11.1	100% (89.4–100)	96.6% (93.6–98.4)	78.6	100
		7	102	9	1	91	1	9.8	90.0% (55.5–99.7)	98.9% (94.1–100)	90.0	98.9
		8	50	3	1	46	0	6.0	100% (29.2–100)	97.9% (88.7–99.9)	75.0	100
		ALL	1445	170	41	1228	6	12.2	96.6% (92.7–98.7)	96.8% (95.6–97.7)	80.6	99.5

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

Table 6c: Aptima Combo 2 Assay PreservCyt Specimens vs. Patient Infected Status

Site	AC2/CT PreservCyt Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
1	Positive	14	0	0	2	14.0	100% (76.8 - 100)	97.7% (91.9 - 99.7)	87.5	100
	Negative	0	0	1	83					
	Total	14	0	1	85					
2	Positive	4	0	0	0	3.2	100% (39.8 - 100)	100% (97.0 - 100)	100	100
	Negative	0	0	2	118					
	Total	4	0	2	118					
3	Positive	29	0	0	2	6.5	93.5% (78.6 - 99.2)	99.5% (98.4 - 99.9)	93.5	99.5
	Negative	2	0	2	440					
	Total	31	0	2	442					
4	Positive	8	1	0	4	2.8	100% (63.1 - 100)	98.2% (95.9 - 99.4)	61.5	100
	Negative	0	2	1	271					
	Total	8	3	1	275					
5	Positive	13	0	0	2	4.7	92.9% (66.1 - 99.8)	99.3% (97.5 - 99.9)	86.7	99.6
	Negative	1	1	4	276					
	Total	14	1	4	278					
6	Positive	19	0	0	1	5.2	100% (82.4 - 100)	99.7% (98.4 - 100)	95.0	100
	Negative	0	1	6	337					
	Total	19	1	6	338					
All	Positive	87	1	0	11	5.5	96.7% (90.6 - 99.3)	99.2% (98.7 - 99.6)	87.9	99.8
	Negative	3	4	16	1525					
	Total	90	5	16	1536					

+/+ = Positive Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the ACT assay.
+/- = Positive Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the ACT assay.
-/+ = Negative Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the ACT assay.
-/- = Negative Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the ACT assay.

Chlamydia trachomatis Analysis for Female Patient Infected Status

Table 7a: Endocervical Swab and Urine Specimen

Patient Infected Status	NAAT 1		NAAT 2		Aptima Combo 2 Assay		Symptom Status	
	FU	FS	FU	FS	FU	FS	Sympt	Asympt
Infected	NA	NA	+	+	+	+	1	0
Infected	NA	+	NA	+	+	+	1	0
Infected	NA	+	+	+	-	+	0	1
Infected	-	+	NA	+	-	+	1	0
Infected	-	+	-	+	-	+	4	0
Infected	-	+	-	+	+	+	6	1
Infected	-	+	+	+	-	+	1	0
Infected	-	+	+	+	+	+	7	3
Infected	+	NA	+	+	+	+	1	0
Infected	+	-	NA	+	+	-	1	0
Infected	+	-	+	-	-	-	1	0
Infected	+	-	+	-	+	-	7	1
Infected	+	-	+	-	+	+	2	1
Infected	+	-	+	+	+	-	1	0
Infected	+	-	+	+	+	+	3	3
Infected	+	+	NA	+	+	+	6	2
Infected	+	+	-	NA	+	+	1	0
Infected	+	+	-	+	+	+	7	3
Infected	+	+	+	NA	+	+	1	0
Infected	+	+	+	-	+	+	2	2
Infected	+	+	+	+	-	-	1	0
Infected	+	+	+	+	-	+	1	1
Infected	+	+	+	+	+	NA	1	0
Infected	+	+	+	+	+	+	88	44
Non-infected	-	-	-	-	NA	-	1	1
Non-infected	-	-	-	-	-	NA	2	1
Non-infected	-	-	-	-	-	-	648	497
Non-infected	-	-	-	-	-	+	18	4
Non-infected	-	-	-	-	+	-	4	3
Non-infected	-	-	-	-	+	+	4	2
Total							822	570

FU = Female Urine; **FS** = Female Endocervical swab.

"NA" represents specimen not obtained or available for testing.

Table 7b: Patient-Collected and Clinician-Collected Vaginal Swab Specimen

Patient Infected Status	NAAT 1		NAAT 2 (Aptima Combo 2)		Aptima Combo 2 Assay		Symptom Status		Total
	FS	FU	FS	FU	PVS	CVS	Symp	Asymp	
Infected	+	+	+	+	+	+	79	43	122
Infected	+	+	+	+	+	-	0	1	1
Infected	+	+	+	+	-	+	1	0	1
Infected	+	+	+	+	NA	-	1	0	1
Infected	+	-	+	+	+	+	8	5	13
Infected	+	-	+	+	-	-	1	0	1
Infected	+	-	+	+	NA	+	1	0	1
Infected	+	=	+	+	+	+	1	0	1
Infected	-	+	+	+	+	+	8	3	11
Infected	-	+	+	+	-	-	1	0	1
Infected	-	-	+	+	+	+	1	2	3
Infected	-	NA	+	+	+	+	1	0	1
Infected	+	+	+	-	+	+	5	3	8
Infected	+	-	+	-	+	+	5	0	5
Infected	+	-	+	-	-	+	2	0	2
Infected	+	+	-	+	+	+	0	1	1
Infected	-	+	-	+	+	+	1	4	5
Infected	-	+	-	+	+	-	1	0	1
Infected	-	+	-	+	-	-	0	1	1
Non-infected	-	-	+	-	+	+	0	4	4
Non-infected	-	-	+	-	+	-	2	1	3
Non-infected	-	-	+	-	-	+	2	1	3
Non-infected	-	-	+	-	-	-	6	4	10
Non-infected	-	-	+	-	NA	+	1	0	1
Non-infected	-	-	+	-	NA	-	1	0	1
Non-infected	-	-	-	+	+	+	4	2	6
Non-infected	-	-	-	+	+	-	1	0	1
Non-infected	-	-	-	+	-	-	0	2	2
Non-infected	+	-	-	-	-	-	1	1	2
Non-infected	-	+	-	-	-	-	1	2	3
Non-infected	-	-	-	-	+	+	3	2	5
Non-infected	-	-	-	-	+	-	2	7	9
Non-infected	-	-	-	-	-	+	12	3	15
Non-infected	-	-	-	-	-	-	623	516	1139
Non-infected	-	-	-	-	-	NA	0	2	2
Non-infected	-	-	-	-	-	=	1	0	1
Non-infected	-	-	-	-	NA	+	0	1	1
Non-infected	-	-	-	-	NA	-	11	8	19
Non-infected	-	-	-	-	NA	NA	1	0	1
Non-infected	-	-	-	-	NA	=	0	1	1
Non-infected	-	-	-	-	=	+	0	1	1
Non-infected	-	NA	-	-	-	-	2	2	4
Non-infected	-	NA	-	-	NA	-	0	1	1
Non-infected	-	=	-	-	-	-	12	9	21
Non-infected	-	=	-	-	-	NA	0	1	1
Non-infected	=	-	-	-	-	-	1	1	2

Table 7b: Patient-Collected and Clinician-Collected Vaginal Swab Specimen (continued)

Patient Infected Status	NAAT 1		NAAT 2 (Aptima Combo 2)		Aptima Combo 2 Assay		Symptom Status		Total
	FS	FU	FS	FU	PVS	CVS	Symp	Asymp	
Non-infected	-	-	-	NA	-	-	0	1	1
Non-infected	-	-	NA	-	-	-	5	4	9
Non-infected	-	-	=	-	-	+	1	0	1
Non-infected	-	-	=	-	-	-	1	0	1
Total							811	640	1451

FS = Female Endocervical swab; **FU** = Female Urine; **PVS** = Asymptomatic Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab. "NA" represents specimen not obtained or available for testing. The equal symbol (=) represents equivocal on repeat testing.

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

Patient Infected Status	Endocervical Swab Result		Symptom Status	
	AC2	ACT	Symp	Asymp
Infected	+	+	30	60
Non-Infected	-	+	4	12
Non-Infected	+	-	3	2
Non-Infected	-	-	322	1214
Total			359	1288

C. trachomatis Analysis for Male Patient Infected Status

Table 8: C. trachomatis Urethral Swab and Urine Specimen Analysis for Male Patient Infected Status

Patient Infected Status	NAAT 1		NAAT 2	Aptima Combo 2 Assay		Symptom Status	
	MU	MS	MU	MU	MS	Sympt	Asympt
Infected	NA	+	+	+	+	2	0
Infected	-	+	+	+	+	10	4
Infected	+	NA	+	+	NA	4	6
Infected	+	NA	+	+	-	2	0
Infected	+	NA	+	+	+	21	1
Infected	+	-	+	+	-	3	3
Infected	+	-	+	+	+	4	3
Infected	+	+	NA	-	+	1	0
Infected	+	+	NA	+	+	8	2
Infected	+	+	-	+	+	12	4
Infected	+	+	+	-	-	1	0
Infected	+	+	+	-	+	1	3
Infected	+	+	+	+	NA	1	0
Infected	+	+	+	+	-	1	1
Infected	+	+	+	+	+	131	53
Non-infected	-	-	-	NA	-	0	2
Non-infected	-	-	-	-	NA	13	8
Non-infected	-	-	-	-	-	461	303
Non-infected	-	-	-	-	+	10	5
Non-infected	-	-	-	+	-	3	4
Non-infected	-	-	-	+	+	5	0
Total						694	402

MU = Male Urine; **MS** = Male Urethral Swab.

"NA" represents specimen not obtained or available for testing.

Neisseria gonorrhoeae Performance Tables

N. gonorrhoeae Sensitivity and Specificity

Table 9a: Aptima Combo 2 Assay Specimens vs. Patient Infected Status

Specimen		Symptoms	N	TP	FP[4]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Male	Swab	Sympt	724	304	5[a]	412	3	99.0% (97.2–99.8)	98.8% (97.2–99.6)
		Asympt	378	15	12[b]	351	0	100% (78.2–100)	96.7% (94.3–98.3)
		All[1]	1103	319	17[c]	764	3	99.1% (97.3–99.8)	97.8% (96.5–98.7)
	Urine	Sympt	750	311	1[d]	433	5	98.4% (96.3–99.5)	99.8% (98.7–100)
		Asympt	383	13	2[e]	368	0	100% (75.3–100)	99.5% (98.1–99.9)
		All[1]	1134	324	3[f]	802	5	98.5% (96.5–99.5)	99.6% (98.9–99.9)
Female	Swab	Sympt	881	94	15[g]	772	0	100% (96.2–100)	98.1% (96.9–98.9)
		Asympt	596	31	2[h]	562	1	96.9% (83.8–99.9)	99.6% (98.7–100)
		All[2]	1479	126	17[i]	1335	1	99.2% (95.7–100)	98.7% (98.0–99.3)
	Urine	Sympt	883	87	7[j]	782	7	92.6% (85.3–97.0)	99.1% (98.2–99.6)
		Asympt	599	28	3[k]	564	4	87.5% (71.0–96.5)	99.5% (98.5–99.9)
		All[2]	1484	116	10[l]	1347	11	91.3% (85.0–95.6)	99.3% (98.6–99.6)
Total	Swab	Sympt	1605	398	20[m]	1184	3	99.3% (97.8–99.8)	98.3% (97.4–99.0)
		Asympt	974	46	14[n]	913	1	97.9% (88.7–99.9)	98.5% (97.5–99.2)
		All[3]	2582	445	34[o]	2099	4	99.1% (97.7–99.8)	98.4% (97.8–98.9)
	Urine	Sympt	1633	398	8[p]	1215	12	97.1% (94.9–98.5)	99.3% (98.7–99.7)
		Asympt	982	41	5[q]	932	4	91.1% (78.8–97.5)	99.5% (98.8–99.8)
		All[3]	2618	440	13[r]	2149	16	96.5% (94.4–98.0)	99.4% (99.0–99.7)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

[1]Includes 1 male subject for whom symptoms were not reported.

[2] Includes 1 female for whom symptoms were not reported.

[3] Includes 1 male and 1 female for whom symptoms were not reported.

[4] GC Alternate TMA results represents # positive results/# specimens tested: a: 5/5, b: 12/12, c: 17/17, d: 0/1, e: 2/2, f: 2/3, g: 13/15, h: 2/2, i: 15/17, j: 4/7, k: 0/2, l: 4/9, m: 18/20, n: 14/14, o: 32/34, p: 4/8, q: 2/4, and r: 6/12.

Table 9b: Aptima Combo 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Symptom Status	N	TP	FP[1]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Patient-Collected	Vaginal Swab	Asympt	629	21	3[a]	605	0	100% (83.9–100)	99.5% (98.6–99.9)
Clinician-Collected	Vaginal Swab	Sympt	807	51	7[b]	747	2	96.2% (87.0–99.5)	99.1% (98.1–99.6)
		Asympt	637	21	4[c]	611	1	95.5% (77.2–99.9)	99.3% (98.3–99.8)
		All	1444	72	11[d]	1358	3	96.0% (88.8–99.2)	99.2% (98.6–99.6)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

[1]GC TMA Alternate Amplification results represents # positive results/# specimens tested: a: 3/3, b: 6/7, c: 3/4, and d: 9/11.

Table 9c: Aptima Combo 2 Assay PreservCyt Specimens vs. Patient Infected Status

Symptom Status	AC2/GC PreservCyt Result	+/+	+/-	-/+	-/-	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Asympt	Positive	5	0	1[1]	3		
	Negative	1	0	5	1273	83.3% (35.9 - 99.6)	99.7% (99.2 - 99.9)
	Total	6	0	6	1276		
Sympt	Positive	7	0	0	0		
	Negative	0	0	0	352	100% (59.0 - 100)	100% (99.0 - 100)
	Total	7	0	0	352		
All	Positive	12	0	1	3		
	Negative	1	0	5	1625	92.3% (64.0 - 99.8)	99.8% (99.4 - 99.9)
	Total	13	0	6	1628		

[1] One specimen had a discordant result: Equivocal endocervical swab specimen result in the Aptima Combo 2 assay/ Positive endocervical swab specimen result in the APTIMA GC assay.

+/+ = Positive Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the AGC assay.

+/- = Positive Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the AGC assay.

-/+ = Negative Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the AGC assay.

-/- = Negative Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the AGC assay.

Neisseria gonorrhoeae Performance by Clinical Site

Table 10a: Aptima Combo 2 Assay Specimens vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Male	Swab	1	159	56	1	101	1	35.8	98.2% (90.6–100)	99.0% (94.7–100)	98.2	99.0
		2	97	13	0	84	0	13.4	100% (75.3–100)	100% (95.7–100)	100	100
		3	264	71	6	187	0	26.9	100% (94.9–100)	96.9% (93.4–98.9)	92.2	100
		4	53	20	0	33	0	37.7	100% (83.2–100)	100% (89.4–100)	100	100
		5	139	12	0	127	0	8.6	100% (73.5–100)	100% (97.1–100)	100	100
		6	336	94	10	231	1	28.3	98.9% (94.3–100)	95.9% (92.5–98.0)	90.4	99.6
		7	55	53	0	1	1	98.2*	98.1% (90.1–100)	100% (2.5–100)	100	50.0
		ALL	1103	319	17	764	3	29.2	99.1% (97.3–99.8)	97.8% (96.5–98.7)	94.9	99.6
	Urine	1	161	57	0	103	1	36.0	98.3% (90.8–100)	100% (96.5–100)	100	99.0
		2	104	19	0	85	0	18.3	100% (82.4–100)	100% (95.8–100)	100	100
		3	265	71	2	192	0	26.8	100% (94.9–100)	99.0% (96.3–99.9)	97.3	100
		4	53	20	0	33	0	37.7	100% (83.2–100)	100% (89.4–100)	100	100
		5	160	14	0	146	0	8.8	100% (76.8–100)	100% (97.5–100)	100	100
		6	335	89	1	241	4	27.8	95.7% (89.4–98.8)	99.6% (97.7–100)	98.9	98.4
		7	56	54	0	2	0	96.4*	100% (93.4–100)	100% (15.8–100)	100	100
		ALL	1134	324	3	802	5	29.0	98.5% (96.5–99.5)	99.6% (98.9–99.9)	99.1	99.4
Female	Swab	1	196	30	2	164	0	15.3	100%(88.4–100)	98.8% (95.7–99.9)	93.8	100
		2	83	9	1	72	1	12.0	90.0% (55.5–99.7)	98.6% (92.6–100)	90.0	98.6
		3	191	31	2	158	0	16.2	100% (88.8–100)	98.8% (95.6–99.8)	93.9	100
		4	215	7	0	208	0	3.3	100% (59.0–100)	100% (98.2–100)	100	100
		5	382	8	1	373	0	2.1	100% (63.1–100)	99.7% (98.5–100)	88.9	100
		6	278	36	8	234	0	12.9	100% (90.3–100)	96.7% (93.6–98.6)	81.8	100
		7	134	5	3	126	0	3.7	100% (47.8–100)	97.7% (93.4–99.5)	62.5	100
		ALL	1479	126	17	1335	1	8.6	99.2% (95.7–100)	98.7% (98.0–99.3)	88.1	99.9
	Urine	1	196	24	2	164	6	15.3	80.0% (61.4–92.3)	98.8% (95.7–99.9)	92.3	96.5
		2	83	9	1	72	1	12.0	90.0% (55.5–99.7)	98.6% (92.6–100)	90.0	98.6
		3	191	30	2	158	1	16.2	96.8% (83.3–99.9)	98.8% (95.6–99.8)	93.8	99.4
		4	215	5	2	206	2	3.3	71.4% (29.0–96.3)	99.0% (96.6–99.9)	71.4	99.0
		5	383	8	0	375	0	2.1	100% (63.1–100)	100% (99.0–100)	100	100
		6	282	35	2	244	1	12.8	97.2% (85.5–99.9)	99.2% (97.1–99.9)	94.6	99.6
		7	134	5	1	128	0	3.7	100% (47.8–100)	99.2% (95.8–100)	83.3	100
		ALL	1484	116	10	1347	11	8.6	91.3% (85.0–95.6)	99.3% (98.6–99.6)	92.1	99.2

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

* Prevalence over-estimated due to initial collection being limited to screening for symptomatic subjects.

Table 10b: Aptima Combo 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Patient-Collected	Vaginal Swab	1	70	5	1	65	0	7.1	100% (47.8 - 100)	98.5 (91.7 - 100)	83.3	100
		2	46	7	0	39	0	15.2	100% (59.0 - 100)	100% (91.0 - 100)	100	100
		3	45	2	0	43	0	4.4	100% (15.8 - 100)	100% (91.8 - 100)	100	100
		4	152	1	0	151	0	0.7	100% (2.5 - 100)	100% (97.6 - 100)	100	100
		5	130	1	0	129	0	0.8	100% (2.5 - 100)	100% (97.2 - 100)	100	100
		6	75	5	2	68	0	6.7	100% (47.8 - 100)	97.1 (90.1 - 99.7)	71.4	100
		7	68	0	0	68	0	0.0	N/A	100% (94.7 - 100)	N/A	100
		8	43	0	0	43	0	0.0	N/A	100% (91.8 - 100)	N/A	100
		ALL	629	21	3	605	0	3.3	100% (83.9 - 100)	99.5 (98.6 - 99.9)	87.5	100
Clinician-Collected	Vaginal Swab	1	227	12	3	212	0	5.3	100% (73.5 - 100)	98.6% (96.0 - 99.7)	80.0	100
		2	196	31	2	163	0	15.8	100% (88.8 - 100)	98.8% (95.7 - 99.9)	93.9	100
		3	113	3	0	109	1	3.5	75.0% (19.4 - 99.4)	100% (96.7 - 100)	100	99.1
		4	262	5	2	255	0	1.9	100% (47.8 - 100)	99.2% (97.2 - 99.9)	71.4	100
		5	198	2	0	196	0	1.0	100% (15.8 - 100)	100% (98.1 - 100)	100	100
		6	296	18	4	272	2	6.8	90.0% (68.3 - 98.8)	98.6% (96.3 - 99.6)	81.8	99.3
		7	102	0	0	102	0	0.0	N/A	100% (96.4 - 100)	N/A	100
		8	50	1	0	49	0	2.0	100% (2.5 - 100)	100% (92.7 - 100)	100	100
		ALL	1444	72	11	1358	3	5.2	96.0% (88.8 - 99.2)	99.2% (98.6 - 99.6)	86.7	99.8

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

Table 10c: Aptima Combo 2 Assay PreservCyt Specimens vs. Patient Infected Status

Site	AC2/GC PreservCyt Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
1	Positive	5	0	0	0	5.0	100% (47.8 - 100)	100% (96.2 - 100)	100	100
	Negative	0	0	0	95					
	Total	5	0	0	95					
2	Positive	1	0	0	0	0.8	100% (2.5 - 100)	100% (97.0 - 100)	100	100
	Negative	0	0	0	123					
	Total	1	0	0	123					
3	Positive	4	0	0	0	1.1	80.0% (28.4 - 99.5)	100% (99.2 - 100)	100	99.8
	Negative	1	0	0	470					
	Total	5	0	0	470					
4	Positive	1	0	0	0	0.3	100% (2.5 - 100)	100% (98.7 - 100)	100	100
	Negative	0	0	3	283					
	Total	1	0	3	283					
5	Positive	0	0	0	3	0.0	N/A	99.0% (97.1 - 99.8)	0.0	100
	Negative	0	0	0	294					
	Total	0	0	0	297					
6	Positive	1	0	1[1]	0	0.3	100% (2.5 - 100)	99.7% (98.5 - 100)	50.0	100
	Negative	0	0	2	360					
	Total	1	0	3	360					
All	Positive	12	0	1	3	0.8	92.3% (64.0 - 99.8)	99.8% (99.4 - 99.9)	75.0	99.9
	Negative	1	0	5	1625					
	Total	13	0	6	1628					

[1] One specimen had a discordant result: Equivocal endocervical swab specimen result in the Aptima Combo 2 assay/Positive endocervical swab specimen result in the APTIMA GC assay.

+/+ = Positive Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the AGC assay.

+/- = Positive Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the AGC assay.

-/+ = Negative Endocervical Swab Specimen Result in the AC2 assay / Positive Endocervical Swab Specimen Result in the AGC assay.

-/- = Negative Endocervical Swab Specimen Result in the AC2 assay / Negative Endocervical Swab Specimen Result in the AGC assay.

Neisseria gonorrhoeae Analysis for Female Patient Infected Status

Table 11a: Endocervical Swab and Urine Specimen

Patient Infected Status	NAAT		Culture	Aptima Combo 2 Assay		Symptom Status	
	FU	FS	FS	FU	FS	Symp	Asymp
Infected	NA	+	+	+	+	1	1
Infected	-	-	+	-	-	0	1
Infected	-	+	+	-	+	5	2
Infected	-	+	+	+	+	9	2
Infected	+	NA	+	+	+	1	0
Infected	+	-	+	+	+	3	1
Infected	+	+	NA	+	+	0	1
Infected	+	+	-	+	+	11	2
Infected	+	+	+	-	+	2	1
Infected	+	+	+	+	+	62	21
Non-Infected	-	-	-	-	NA	2	3
Non-Infected	-	-	-	-	-	768	559
Non-Infected	-	-	-	-	+	12	2
Non-Infected	-	-	-	+	-	4	3
Non-Infected	-	-	-	+	+	3	0
Total						883	599

FU = Female Urine; **FS** = Female Endocervical Swab.
"NA" represents specimen not obtained or available for testing.

Table 11b: Patient-Collected and Clinician-Collected Vaginal Swab Specimen Analysis

Patient Infected Status	NAAT 1		NAAT 2		Aptima Combo 2 Assay		Symptom Status		Total
	FS	FU	FS	FU	PVS	CVS	Sympt	Asympt	
Infected	+	+	+	+	+	+	44	15	59
Infected	+	+	+	+	+	-	1	0	1
Infected	+	+	+	+	NA	+	0	1	1
Infected	+	-	+	+	+	+	2	2	4
Infected	+	NA	+	+	+	+	1	0	1
Infected	-	+	+	+	+	+	1	1	2
Infected	-	-	+	+	+	+	1	1	2
Infected	+	+	+	-	+	+	1	0	1
Infected	+	-	+	-	+	+	1	1	2
Infected	+	-	+	-	+	-	1	0	1
Infected	+	+	-	+	+	+	1	0	1
Infected	-	+	-	+	+	+	0	1	1
Infected	-	+	-	+	+	-	0	1	1
Infected	+	+	-	-	-	+	1	0	1
Non-infected	-	-	+	-	-	-	5	1	6
Non-infected	-	-	-	+	-	-	1	0	1
Non-infected	+	-	-	-	+	+	1	0	1
Non-infected	+	-	-	-	-	-	5	2	7
Non-infected	-	+	-	-	+	+	0	1	1
Non-infected	-	+	-	-	-	-	2	1	3
Non-infected	-	-	-	-	+	+	2	0	2
Non-infected	-	-	-	-	+	-	1	1	2
Non-infected	-	-	-	-	-	+	2	2	4
Non-infected	-	-	-	-	-	-	698	577	1275
Non-infected	-	-	-	-	-	NA	0	2	2
Non-infected	-	-	-	-	-	=	2	0	2
Non-infected	-	-	-	-	NA	-	15	9	24
Non-infected	-	-	-	-	NA	NA	1	0	1
Non-infected	-	NA	-	-	-	-	2	2	4
Non-infected	-	NA	-	-	NA	-	0	1	1
Non-infected	-	=	-	-	-	-	11	10	21
Non-infected	-	=	-	-	-	NA	0	1	1
Non-infected	=	-	-	-	-	-	1	1	2
Non-infected	-	-	-	NA	-	-	0	1	1
Non-infected	-	-	NA	-	-	-	5	4	9
Non-infected	-	-	=	-	-	-	1	1	2
Total							810	640	1450

FS = Female Endocervical swab; **FU** = Female Urine; **PVS** = Asymptomatic Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab; "NA" represents specimen not obtained or available for testing. The equal symbol (=) represents equivocal on repeat testing.

N. gonorrhoeae Analysis for Female Patient Infected Status

Table 11c: PreservCyt Solution Liquid Pap Specimen Clinical Study Patient Infected Status Results for N. gonorrhoeae

Patient Infected Status	Endocervical Swab Result		Symptom Status	
	AC2	AGC	Symp	Asymp
Infected	+	+	7	6
Non-Infected	=	+	0	1
Non-Infected	-	+	0	5
Non-Infected	-	-	352	1276
Total			359	1288

N. gonorrhoeae Analysis for Male Patient Infected Status

Table 12: Urethral Swab and Urine Specimen

Patient Infected Status	NAAT 1		Culture	Aptima Combo 2 Assay		Symptom Status	
	MU	MS	MS	MU	MS	Symp	Asymp
Infected	NA	+	+	+	+	1	0
Infected	-	NA	+	NA	+	0	1
Infected	-	NA	+	+	+	1	0
Infected	-	-	+	-	-	1	0
Infected	-	+	+	+	+	4	1
Infected	+	NA	+	NA	+	0	1
Infected	+	NA	+	+	NA	8	0
Infected	+	NA	+	+	-	1	0
Infected	+	NA	+	+	+	50	1
Infected	+	-	+	+	+	4	1
Infected	+	+	NA	+	+	1	0
Infected	+	+	-	+	+	11	1
Infected	+	+	+	-	-	1	0
Infected	+	+	+	-	+	3	0
Infected	+	+	+	+	NA	1	0
Infected	+	+	+	+	+	229	9
Non-infected	-	-	-	NA	-	0	1
Non-infected	-	-	-	NA	+	0	1
Non-infected	-	-	-	-	NA	17	9
Non-infected	-	-	-	-	-	411	349
Non-infected	-	-	-	-	+	5	10
Non-infected	-	-	-	+	-	1	1
Non-infected	-	-	-	+	+	0	1
Total						750	387

MU = Male Urine; **MS** = Male Urethral Swab; NA = Specimen not obtained or available for testing.

RLU Distribution of Aptima Controls

The distribution of the RLUs for the Aptima Positive Control, GC / Negative Control, CT and the Aptima Positive Control, CT / Negative Control, GC from all the Aptima Combo 2 assay runs performed during the clinical specimen studies is presented in Table 13.

Table 13: Distribution of Total RLU of the Aptima Combo 2 Assay Controls

Control	Statistics	Total RLU (x 1000)		
		Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study	Vaginal Swab Specimen Clinical Study	PreservCyt Solution Liquid Pap Specimen Clinical Study
Positive Control, CT/Negative Control, GC	Maximum	1572	1996	1747
	75th Percentile	1160	1279	1264
	Median	1063	1135	1165
	25th Percentile	996	933	1024
	Minimum	274	174	494
Positive Control, GC/Negative Control, CT	Maximum	1359	1420	1438
	75th Percentile	1202	1255	1288
	Median	1093	1169	1201
	25th Percentile	989	1084	1099
	Minimum	167	249	166

Precision Study

Precision testing was performed at three sites to obtain measures of repeatability and reproducibility. Precision studies were conducted as part of the Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study and the PreservCyt Solution liquid Pap Specimen Clinical Study. For the former study, each site was provided with three identical panels of 13 samples containing 0 to 500 fg of CT rRNA, 0 to 25,000 fg of GC rRNA, or combinations of both CT and GC rRNA. Testing was performed over three days using a different assay kit lot each day. The overall RLU, within-run, between-run, and between-site descriptive statistics are summarized in Table 14a.

For the latter precision study, reproducibility was established with a 12-member panel generated by spiking PreservCyt Solution with 0 to 2,000 fg/assay of CT and 0 to 5,000 fg/assay of GC rRNA and aliquotting 1.0 mL into the Aptima Specimen Transfer Kit collection tube. Two (2) operators at each of the three sites performed one run per day on each of three days, totaling three valid runs per operator. Testing was performed using one assay kit lot. The results of this precision study are summarized in Table 14b.

For both studies, reproducibility was established by spiking the appropriate transport medium (STM, PreservCyt Solution) with rRNA. Reproducibility when testing swab, urine, or PreservCyt Solution liquid Pap clinical specimens containing target organism has not been determined.

Table 14a: Swab Transport Medium

Panel Member		N	Mean RLU (x1000)	Within-Run		Between-Run		Between-Site	
				SD (RLU)	CV (%)	SD (RLU)	CV (%)	SD (RLU)	CV (%)
High	CT Swab	54	1,055	76,588	7.3	83,711	7.9	150,332	14.2
	Dual Swab*	54	2,338	93,449	4.0	90,317	3.9	142,898	6.1
	Dual Urine*	54	2,281	91,487	4.0	106,715	4.7	152,747	6.7
	GC Swab	54	1,265	30,561	2.4	55,642	4.4	34,413	2.7
Mid	CT Swab	54	1,001	69,831	7.0	77,701	7.8	159,774	16.0
	Dual Swab*	54	2,241	152,377	6.8	58,353	2.6	139,983	6.2
	GC Swab	54	1,249	35,142	2.8	60,638	4.9	46,364	3.7
Low	CT Swab	54	1,013	61,795	6.1	90,906	9.0	131,207	13.0
	Dual Swab*	54	2,085	286,034	13.7	161,764	7.8	58,837	2.8
	Dual Urine*	54	2,201	95,705	4.3	118,760	5.4	106,802	4.9
	GC Swab	54	1,177	42,478	3.6	69,821	5.9	29,836	2.5
Negative	Swab	54	7	1,301	18.3	2,311	32.5	1,901	26.8
	Urine	54	7	861	12.0	2,299	32.1	1,994	27.9

* Dual positive panel members contained both CT and GC rRNA.

Table 14b: PreservCyt Solution

Concentration (fg/assay)		N	Agreement	Mean RLU (x1000)	Within-Run		Between-Run		Between-Site		Between-Operator	
CT	GC				SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
0	0	162	97.5%	9.7	31.6	N/A	3.4	N/A	6.4	N/A	4.7	N/A
0	5,000	54	96.3%	1296	146	11.3	54.8	4.2	0.0	0.0	0.0	0.0
2,000	0	54	100%	1140	54.1	4.7	79.8	7.0	101	8.9	2.4	0.2
2,000	5,000	54	100%	2345	79.6	3.4	78.0	3.3	94.7	4.0	37.9	1.6
0	250	54	100%	953	114	12.0	0.0	0.0	161	16.9	90.7	9.5
5	0	54	100%	971	58.3	6.0	71.7	7.4	22.8	2.4	85.0	8.8
1,000	2,500	54	100%	2294	114	5.0	88.9	3.9	153	6.7	0.0	0.0
100	250	54	98.1%	1911	139	7.3	130	6.8	348	18.2	39.7	2.1
5	5,000	54	100%	2136	113	5.3	130	6.1	98.8	4.6	166	7.8
2,000	250	54	96.3%	2044	138	6.7	169	8.3	360	17.6	26.9	1.3

RLU - Relative Light Units; **SD** = Standard Deviation; **CV** = Coefficient of Variation; N/A represents specimen not applicable for negative panel members.

Samples with discordant and equivocal results were included in the signal variability analysis.

For CV and SD values equal to 0.0, the variability due to this source is very small relative to other sources of variation.

Aptima Combo 2 Analytical Performance

Note: *The following results were generated with the original version of the Aptima Combo 2 assay using the DTS systems.*

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

Chlamydia trachomatis analytical sensitivity (limits of detection) was determined by directly comparing dilutions of CT organisms in cell culture and in the assay. The analytical sensitivity claim for the assay is one Inclusion-Forming Unit (IFU) per assay (7.25 IFU/swab, 5.0 IFU/mL urine, 9.75 IFU/mL PreservCyt Solution liquid Pap) 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 1.0 IFU/assay of all serovars tested positive in the Aptima Combo 2 assay.

Neisseria gonorrhoeae analytical sensitivity was determined by directly comparing dilutions of 57 different clinical isolates in culture and in the Aptima Combo 2 assay with swab and urine specimens and 20 clinical isolates with PreservCyt Solution liquid Pap specimens. The analytical sensitivity claim for the assay is 50 cells/assay (362 cells/swab, 250 cells/mL urine, 488 cells/mL PreservCyt Solution liquid Pap). However, all strains tested were positive at less than 50 cells/assay.

Analytical Specificity

A total of 198 organisms were evaluated using the Aptima Combo 2 assay in two studies. An initial study included 154 culture isolates which contained 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. An additional study for extragenital samples, included 44 microbes that may be found on the extragenital swabs. The tested organisms included bacteria, fungi, yeast, parasites, and viruses.

In the initial study, all organisms except *C. psittaci, C. pneumoniae*, and the viruses were tested at 1.0 x 10^6 cells/assay in both swab and urine transport medium. The Chlamydia and Neisseria organisms were tested in PreservCyt solution medium. *C. psittaci* and *C. pneumoniae* were tested at 1.0 x 10^5 IFU/assay. The viruses were tested as follows: (a) herpes simplex viruses I and II: 2.5 x 10^4 $TCID_{50}$/assay, (b) human papilloma virus 16: 2.9 x 10^6 DNA copies/assay and (c) cytomegalovirus: 4.8 x 10^5 infected cell culture cells/assay.

In the second study, all organisms were tested in STM. All non-viral isolates were tested at 1.0 x 10^6 CFU/mL except *Bacteriodes oralis*, *Fusobacterium necrophorum* and *Peptostreptococcus micros* which were tested at 1.0 x 10^6 RNA copies/mL. The viruses were tested at 1.0 x 10^5 $TCID_{50}$/mL except for Norovirus group II: 1.0 x 10^6 $TCID_{50}$/mL, Enterovirus Type 68: 1.0 x 10^4 $TCID_{50}$/mL and Infuenza viruses which were tested at 2.0 x 10^3 $TCID_{50}$/mL. Only CT and GC samples produced positive results in the Aptima Combo 2 assay. The list of organisms tested in the first study are shown in Table 15 and the organisms tested in the second study are shown in Table 16.

Table 15: 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)” represents the number of strains tested.

All organisms tested produced a negative result in the Aptima Combo 2 assay based on kinetic profile type and RLU.

Table 16: Cross-Reactivity Microorganisms for Throat and Rectal Specimens

Organism	Organism	Organism
Adenovirus	*Eggerthella lenta*	Metapneumo virus
Anaercoccus spp.	*Entamoeba histolytica*	*Moraxella catarrhalis*
Arcanobacterium haemolyticum	Enterovirus	*Mycoplasma pneumoniae*
Bacteroides oralis	Epstein-Barr Virus	Norovirus
Bordetella parapertussis	*Fusobacterium necrophorum*	*Peptostreptococcus micros*
Bordetella pertussis	*Giardia lamblia*	*Prevotella spp.*
Burkholderia cepacia	*Haemophilus parahaemolyticus*	Respiratory syncytial virus
Campylobacter rectus	*Haemophilus parainfluenzae*	Rhinovirus
Citrobacter koseri	*Helicobacter pylori*	*Shigella dysenteriae*
Clostridium difficile	Hepatitis B Virus	*Shigella flexneri*
Coronavirus	Hepatitis C Virus	*Shigella sonnei*
Corynebacterium diphtheriae	Human influenza virus A	*Stenotrophomonas maltophilia*
Corynebacterium pseudodiphtheriticum	Human influenza virus B	*Streptococcus anginosus group*
Coxsackie Virus	*Legionella jordanis*	*Veillonella parvula*
Echovirus	*Legionella micdadei*	

Interfering Substances

The following interfering substances were individually spiked into Swab and PreservCyt Solution liquid Pap specimens: 10% blood, contraceptive jelly, spermicide, moisturizer, hemorrhoidal anesthetic, body oil, powder, anti-fungal cream, vaginal lubricants, feminine spray and leukocytes (1.0×10^6 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 (1.0×10^6 cells/mL), cellular debris, vitamins, minerals, acetaminophen, aspirin and ibuprofen. All were tested for potential assay interference in the absence and presence of CT and GC at the estimated rRNA equivalent of 1.0 CT IFU/assay (5 fg/assay) and 50 GC cells/assay (250 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 Combo 2 assay.

Recovery

Escherichia coli and *Gardnerella vaginalis* (2.4×10^5 cells/assay) and *Lactobacillus acidophilus*, *Gardnerella vaginalis*, *Bacteroides ureolyticus* and *Staphylococcus epidermis* (1.0×10^8 cells/assay) were added to samples containing the rRNA equivalent of approximately 1.0 CT IFU (5 fg) and 50 GC cells (250 fg). These additions did not interfere with the amplification and detection of CT or GC rRNA using the Aptima Combo 2 assay.

Specimen Stability Studies

A. Endocervical Swab Specimens

Data to support the recommended shipping and storage conditions for endocervical swab samples were generated with pooled negative swab samples. Five pooled samples were spiked with CT and GC at final concentrations of 10 IFU and 100 CFU per reaction, respectively. The spiked samples were held at -70°C, -20°C, 4°C, and 30°C. Samples were tested in duplicate at days 0, 20, 35, 60, and 90. All test conditions were positive for both CT and GC at all times and temperatures.

B. PreservCyt Solution Liquid Pap Specimens

Data to support the recommended shipping and storage conditions for PreservCyt Solution liquid Pap samples were generated with pooled negative PreservCyt Solution liquid Pap samples. Four pooled samples were spiked with CT and GC at final concentrations of 10 IFU and 100 CFU per reaction, respectively. The PreservCyt Solution liquid Pap samples were placed at 30°C for 7 days, after which 1.0 mL of the sample was added to an Aptima Transfer Tube. The spiked samples were held at 4°C, 10°C and 30°C. Samples stored at 4°C and 10°C were tested in duplicate at days 0, 6, 13, 26, 30 and 36. Samples stored at 30°C were tested in duplicate at days 0, 5, 8, 14 and 17. Four spiked PreservCyt Solution liquid Pap sample pools were added to Aptima Transfer Tubes and placed at 30°C for 14 days before being stored at either -20°C or -70°C. The -20°C samples and the -70°C samples were tested in duplicate after 0, 30, 60, 90 and 106 days of storage. All test conditions were positive for both CT and GC at all times and temperatures.

C. Vaginal Swab Specimens

Data to support the recommended shipping and storage conditions for vaginal swab samples were generated with pooled negative swab samples. Fifteen vaginal swab pools were spiked with CT and GC at final concentrations of 1.0 IFU and 50 CFU per reaction, respectively. The spiked samples were held at -70°C, -20°C, 4°C, and 30°C. Samples were tested using one aliquot at days 0, 20, 36, 73, and 114. All test conditions were positive for both CT and GC at all times and temperatures.

D. Urine Specimens

Data to support the recommended shipping and storage conditions for urine samples were generated with ten female and ten male negative urine samples. The urine samples were spiked with CT and GC at final concentrations of 10 IFU and 100 CFU per reaction, respectively. Two sets of the spiked urine samples were held at 4°C and 30°C for 24 hours prior to being added to the Urine Transport Media (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 were positive for both CT and GC when the urine samples were held at 4°C prior to addition of the UTM. When the urine samples were held at 30°C prior to addition of the UTM, all of the samples were positive for CT and 95% of the samples were positive for GC at Day 35. These same samples were tested after 116 days of storage at -20°C and -70°C. All samples were positive for CT and GC under both storage conditions.

E. Additional Frozen (at -20°C) Specimen Stability Study

Data to support the recommended storage condition at -20°C for endocervical swab, urethral swab, vaginal swab, female urine, male urine, and PreservCyt Solution liquid Pap specimens were generated using 90 specimens for each type with negative result, where 30 specimens were spiked with CT and GC at 1.0 IFU and 50 CFU per reaction, respectively; 30 specimens were spiked at 0.1 IFU and 5 CFU per reaction, respectively; and 30 specimens were unspiked. The specimens were stored at -20°C and were tested at days 0, 200, and 400 days. All spiked specimens met the acceptance criteria of 95% agreement with expected results.

Tigris DTS System Clinical Specimen Agreement

Tigris DTS System Agreement

Agreement between Aptima Combo 2 Assay results generated on the fully automated Tigris DTS system and semi-automated DTS systems was evaluated by testing endocervical swab, male urethral swab, female and male urine, vaginal swab, and PreservCyt Solution liquid Pap specimens. Each of the clinical specimens was tested individually with the Aptima Combo 2 Assay on both the Tigris DTS system and DTS systems at Hologic.

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

Male and female subjects attending STD, urgent care, public health, and family planning clinics were enrolled at seven geographically diverse clinical sites with low to high prevalence for CT and GC. The clinical specimen agreement study evaluated agreement between the two systems using swab and urine specimens from 485 male and 576 female subjects. Of the 1,991 specimens tested, there was a small percentage that initially tested invalid or equivocal for CT or GC on the Tigris DTS system (20, 1.0%) and on the DTS Systems (14, 0.7%). Upon repeat testing, there were two (2) clinical specimens with equivocal GC results on the Tigris DTS system, which are not included in equivalence calculations. Overall percent agreement and percent positive and negative agreements were calculated. Specimens yielding discordant results between the DTS systems and Tigris DTS system were tested in alternate TMA amplification assays for CT and GC, which are nucleic acid amplification tests (NAATs) that target CT or GC rRNA sequences that differ from those targeted in the Aptima Combo 2 Assay. Aptima Combo 2 Assay repeat testing on the DTS Systems was also conducted on specimens yielding discordant Tigris DTS system and DTS system results.

Tables 17 and 18 show the overall percent agreements for all paired test results obtained on the Tigris DTS system and DTS system for swab and urine specimens, respectively. Overall agreements were 98.3% for swab specimens and 99.2% for urine specimens. Refer to Tables 5a and 9a for Aptima Combo 2 performance estimates for endocervical swab, male urethral swab, and female and male urine specimens tested on the DTS systems. Clinical performance estimates for the Tigris DTS system with endocervical swab, male urethral swab, and female and male urine specimens would be expected to be similar given the agreement findings.

Clinical Specimen Agreement Study — Vaginal Swab and PreservCyt Solution Liquid Pap Specimens

Female subjects attending STD, public health, and OB/GYN clinics contributed vaginal swab and PreservCyt Solution liquid Pap specimens. The vaginal swab 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, vaginal swab and PreservCyt Solution liquid Pap specimens were first screened with the Aptima Combo 2 assay on the DTS systems. Specimens with final invalid or equivocal DTS systems results were not selected for further testing on the Tigris DTS system. Aptima Combo 2 assay positive specimens and a subset of Aptima Combo 2 assay negative specimens were selected for comparison testing on the Tigris DTS system. One hundred seventy (170) vaginal swab and 170 PreservCyt Solution liquid Pap specimens from 181 female subjects were tested on both systems. The majority of specimens (110 vaginal swab and 107 PreservCyt Solution liquid Pap specimens) selected for comparison testing were from symptomatic women. Seventeen (17) worklists were initiated: 13 (76.5%) were valid and 4

(23.5%) were invalidated because the instrument detected high background at the luminometer. The instrument had loose Detect 1 and 2 fittings that could have allowed air into the lines or incorrect amounts of detect reagents to be injected. These worklists were valid when retested. Of the 340 specimens tested, none had initial invalid or equivocal test results on the Tigris DTS system.

Tables 19 and 20 show the overall percent agreements for CT and GC detection for all paired test results obtained on the Tigris DTS and DTS systems for vaginal swab and PreservCyt Solution liquid Pap specimens, respectively. Overall agreements were 98.2% for vaginal swab specimens and 98.2% for PreservCyt Solution liquid Pap specimens. Refer to Tables 5b, 5c, 9b, and 9c for Aptima Combo 2 assay performance estimates for vaginal swab and PreservCyt Solution liquid Pap specimens tested on the DTS systems. Clinical performance estimates for the Tigris DTS system with vaginal swab and PreservCyt Solution liquid Pap specimens would be expected to be similar given the agreement findings.

CT/GC Clinical Panel Agreement Study — Endocervical Swab, Male Urethral Swab, and Female and Male Urine Specimens

The CT/GC clinical panel agreement study evaluated equivalence between the two systems using 13 Hologic-prepared CT/GC clinical panels containing 0 to 2,500 Inclusion Forming Units (IFU)/mL of CT and/or 0 to 125,000 Colony Forming Units (CFU)/mL of GC. The CT/GC clinical panels were created from swab and urine specimens collected from 222 male and 117 female subjects who were determined to be non-infected based on negative Aptima Combo 2 Assay swab and urine specimen results on the DTS Systems. Each of the 13 CT/GC panels consisted of 5 replicates of each specimen type (endocervical swab, male urethral swab, female urine, male urine) for a total of 20 replicates per panel.

Table 21 shows the percent agreements with expected CT and GC results for the Tigris DTS system and for the DTS Systems for each of the 13 CT/GC panels. The concentrations ranged from 10 fold below to 1000 fold above the Aptima Combo 2 assay analytical claim limits of 1 IFU/assay for CT and 50 CFU/assay for GC. Also shown in Table 21 is the overall percent agreement (99.3%) between CT/GC panel results from the Tigris DTS system and from the DTS system. Positive and negative agreements are shown in Tables 22 and 23 for CT and GC panel results, respectively. For swab and urine panels, positive agreements were 100% and 96.2% respectively for CT, and were both 100% for GC. Swab and urine negative agreements were 100% and 98.0%, respectively, for CT, and were both 100% for GC. Three of 5 female urine panel replicates, which were one log below the Aptima Combo 2 assay analytical sensitivity claim of 1 IFU/assay for CT, were CT- on the Tigris system. One of 5 female urine panel replicates from a separate panel was CT- on the DTS systems.

Table 17: Clinical Specimen Agreement Study: Endocervical and Male Urethral Swab Specimen Results[1]

Tigris DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**30**	0	0	0	30
CT+/GC-	0	**108**	0	2[5]	110
CT-/GC+	1[2]	0	**67**	0	68
CT-/GC-	0	12[3]	2[4]	**796**	810
Total	31	120	69	798	1018
Percent Agreement (95% C.I.)	96.8% (83.3-99.9)	90.0% (83.2-94.7)	97.1% (89.9-99.6)	99.7% (99.1-100)	n/a
Overall Percent Agreement (95% C.I.): 98.3% (97.3-99.0)					

+ denotes Positive, - denotes Negative,n/a = Not Applicable.

[1]Data not shown: Two specimens tested CT-/GC equivocal on both the Tigris and DTS Systems. One specimen tested CT-/GC- on the Tigris DTS system, but CT-/GC equivocal on the DTS Systems. When retested in the Aptima Combo 2 Assay on the DTS Systems, this specimen tested CT-/GC-. The specimen also tested GC- in the alternate TMA amplification assay.

[2]1/1 was CT+/GC+ when retested on the DTS Systems and was CT+ in the alternate TMA amplification assay.

[3]11/12 were retested. 11/11 were CT-/GC- when retested in the Aptima Combo 2 Assay on the DTS Systems. 9/11 were CT- when tested in the alternate TMA amplification assay and 2/11 were CT+.

[4]2/2 were CT-/GC- when retested in the Aptima Combo 2 Assay on the DTS Systems and were GC- in the alternate TMA amplification assay.

[5]2/2 were CT-/GC- when retested in the Aptima Combo 2 Assay on the DTS Systems and were CT- in the alternate TMA amplification assay.

Table 18: Clinical Specimen Agreement Study: Female and Male Urine Specimen Results

Tigris DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**32**	0	0	0	32
CT+/GC-	0	**100**	0	1[3]	101
CT-/GC+	0	0	**52**	0	52
CT-/GC-	0	8[1]	1[2]	**776**	785
Total	32	108	53	777	970
Percent Agreement (95% C.I.)	100% (89.1-100)	92.6% (85.9-96.7)	98.1% (89.9-100)	99.9% (99.3-100)	n/a
Overall Percent Agreement (95% C.I.): 99.2% (98.1-99.5)					

+ denotes Positive, - denotes Negative, n/a = Not Applicable.

[1] 7/8 were CT-/GC- when retested in the Aptima Combo 2 Assay on the DTS Systems and were CT- in the alternate TMA amplification assay.
1/8 was CT+/GC- when retested in the Aptima Combo 2 Assay on the DTS Systems and was CT+ in the alternate TMA amplification assay.

[2] 1/1 was CT-/GC- when retested in the Aptima Combo 2 Assay on the DTS Systems and was GC- in the alternate TMA amplification assay.

[3] 1/1 was CT-/GC- when retested in the Aptima Combo 2 Assay on the DTS Systems and was CT+ in the alternate TMA amplification assay.

Table 19: Clinical Specimen Agreement Study: Vaginal Swab Specimen Results

Tigris DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**26**	0	0	0	26
CT+/GC-	0	44	0	2	46
CT-/GC+	0	0	**24**	0	24
CT-/GC-	0	0	1	73	74
Total	26	44	25	75	170
Percent Agreement (95% C.I.)	100% (86.8-100)	100% (92.0-100)	96.0% (79.6-99.9)	97.3% (90.7-99.7)	n/a
Overall Percent Agreement (95% CI): 98.2% (94.9-99.6)					

+ denotes Positive, - denotes Negative, n/a = Not Applicable.

Table 20: Clinical Specimen Agreement Study: PreservCyt Solution Liquid Pap Specimen Results

Tigris DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**26**	0	0	0	26
CT+/GC-	0	**44**	0	1	45
CT-/GC+	0	0	**24**	0	24
CT-/GC-	0	1	1	**73**	75
Total	26	45	25	74	170
Percent Agreement (95% C.I.)	100% (86.8-100)	97.8% (88.2-99.9)	96.0% (79.6-99.9)	98.6% (92.7-100)	n/a
Overall Percent Agreement (95% CI): 98.2% (94.9-99.6)					

+ denotes Positive, - denotes Negative, n/a = Not Applicable.

Table 21: CT/GC Clinical Panel Agreement Study: Agreement with Expected CT and GC Results for Endocervical Swab, Male Urethral Swab, and Female and Male Urine Panels

Panel Member CT/GC	Panel Member Concentration[1]		Replicates	CT		GC	
	CT IFU/mL	GC CFU/mL		Tigris %Agrmt	DTS %Agrmt	Tigris %Agrmt	DTS %Agrmt
Low/Low	2.5	125	20	100	100	100	100
Low/High	2.5	125,000	20	100	95[3]	100	100
High/Low	2,500	125	20	100	100	100	100
High/High	2,500	125,000	20	100	100	100	100
Very Low/Neg	0.25[2]	0	20	85[4]	100	100	100
Low/Neg	2.5	0	20	100	100	100	100
Medium/Neg	25	0	20	100	100	100	100
High/Neg	2,500	0	20	100	100	100	100
Neg/Very Low	0	12.5	20	100	100	100	100
Neg/Low	0	125	20	100	100	100	100
Neg/Medium	0	1,250	19	100	100	100	100
Neg/High	0	125,000	20	100	100	100	100
Neg/Neg	0	0	20	100	100	100	100
Overall Percent Agreement between Tigris and DTS (95% C.I.): 99.3% (98.3-99.8)							

IFU = Inclusion Forming Units, CFU = Colony Forming Units, Tigris %Agrmt = Agreement between Tigris with expected results, DTS %Agrmt = Agreement between DTS with expected results.

[1]A collection tube contains approximately 2.9 mL of transport medium for swab specimens and 4.0 mL of transport medium/urine mixture for urine specimens.

[2]The CT concentration in this CT/GC clinical panel member is one log below the Aptima Combo 2 assay analytical sensitivity claim of 1 IFU/assay (7.25 IFU/swab, 5 IFU/mL urine).

[3]One of 5 female urine panel replicates was CT- on the DTS system.

[4]Three of 5 female urine panel replicates were CT- on the Tigris system.

Table 22: CT/GC Clinical Panel Agreement Study: CT Results for the Endocervical and Male Urethral Swab and Female and Male Urine Panels

Specimen	N	DTS+ Tigris+ n	DTS+ Tigris- n	DTS- Tigris+ n	DTS- Tigris- n	Positive Agreement (95% C.I.)	Negative Agreement (95% C.I.)
Swab	129	80	0	0	49	100 (95.5-100)	100 (92.7-100)
Urine	130	76	3[1]	1[2]	50	96.2 (89.3-99.2)	98.0 (89.6-100)

+ denotes Positive, - denotes Negative, C.I. = Confidence Interval.

[1] Three of 5 female urine panel replicates, which were one log below the Aptima Combo 2 Assay analytical sensitivity claim of 1 IFU/assay for CT, were CT- on the Tigris system.

[2] One of 5 female urine panel replicates was CT- on the DTS system.

Table 23: CT/GC Clinical Panel Agreement Study: GC Results for the Endocervical and Male Urethral Swab and Female and Male Urine Panels

Specimen	N	DTS+ Tigris+ n	DTS+ Tigris- n	DTS- Tigris+ n	DTS- Tigris- n	Positive Agreement (95% C.I.)	Negative Agreement (95% C.I.)
Swab	129	79	0	0	50	100 (95.4-100)	100 (92.9-100)
Urine	130	80	0	0	50	100 (95.5-100)	100 (92.9-100)

+ denotes Positive, - denotes Negative, C.I. = Confidence Interval, Tigris = Tigris DTS.

Precision Study

Tigris DTS system precision (i.e., reproducibility) was evaluated at one external clinical site and at Hologic. Aptima Combo 2 assay precision was evaluated across three Tigris DTS Systems, two study sites, two Aptima Combo 2 assay kit lots and four operators. Table 24 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-operator, between-lot, between-run, and within-run variability.

At the external site, two operators performed three worklists (i.e., runs) per Aptima Combo 2 assay kit lot on one Tigris DTS system, completing a total of 6 worklists each. At Hologic, two operators performed three worklists per Aptima Combo 2 Assay kit lot on each of two Tigris DTS system, completing a total of 12 worklists each. Thus, a total of 36 worklists were completed overall. Each worklist was composed of six identical, 12-member precision panels containing 0 to 2,000 fg/assay of CT rRNA and/or 0 to 2,433 fg/assay of GC rRNA. Each worklist was composed of six identical, 12-member precision panels containing 0 to 2,000 fg/assay of CT rRNA and/or 0 to 5,000 fg/assay of GC rRNA. Panel members containing CT and GC were categorized as having low (5 or 100 fg/assay), mid (1000 fg/assay), or high (≥ 2000 fg/assay) concentrations of CT and as having low (≤ 250 fg/assay), mid (approx. 2400 fg/assay), or high (5000 fg/assay) concentrations of GC. Reproducibility was established by spiking swab transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Precision was estimated according to NCCLS Guidelines EP5-A (35).

Table 24: Tigris DTS System Precision Data

Conc.					Within-Run		Between-Site		Between-Lot		Between-Operator		Between-Run	
CT	GC	N	Mean RLU (x1000)	% Agrmt	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)
Neg	Neg	647	4	100	1.25	26.2	0.66	13.9	0.05	1.0	0.08	1.7	0.30	6.4
Neg	High	215	1,216	100	28.5	2.3	61.2	5.0	10.0	0.8	0	0	17.1	1.4
High	Neg	216	1,266	100	38.8	3.0	0	0	93.1	7.3	40.8	3.2	40.4	3.1
High	High	210	2,445	100	54.2	2.2	40.0	1.6	110.3	4.5	28.4	1.1	52.3	2.1
Neg	Low[1]	217	1,132	100	30.3	2.6	61.0	5.3	0	0.0	20.7	1.8	18.5	1.6
Low[1]	Neg	214	1,053	100	72.8	6.9	1.5	0.1	73.8	7.0	28.5	2.7	26.9	2.5
Mid	Mid	214	2,429	100	48.8	2.0	40.0	1.6	101.1	4.1	0	0	52.9	2.1
Low[1]	Low[1]	216	2,112	99.5	112.3	5.3	84.1	3.9	33.2	1.5	34.2	1.6	52.9	2.5
Low[1]	High	216	2,282	100	77.3	3.3	97.8	4.2	59.3	2.6	0	0	41.7	1.8
High	Low[1]	215	2,318	100	61.1	2.6	50.7	2.1	86.2	3.7	4.6	0.2	42.4	1.8

SD = Standard Deviation, %CV = Percent Coefficient of Variation, % Agrmt. = Percent Agreement, Conc. = Concentration.

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 standard deviation and %CV is set to 0. See NCCLS Approved Guidelines EP5-A (35).

[1]Low panel members were spiked at the claimed analytical sensitivities of the assay (5 fg CT rRNA/assay, 250 fg GC rRNA/assay, or both for the dual positive panel member). For CT, the target level tested is the equivalent of approximately 36 fg/swab and 25 fg/mL urine. For GC, the target level tested is the equivalent of approximately 1800 fg/swab and 1250 fg/mL urine. Based on genome size and estimated DNA:RNA ratio/cell of each organism, 5 fg is the equivalent of 1 IFU CT and 250 fg is the equivalent of 50 cells GC.

Tigris DTS System Analytical Performance

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

Analytical Sensitivity Equivalence Study

Dilutions of three CT serovars (E, F, G) associated with urogenital disease were tested on three Tigris DTS system instruments and in parallel on the DTS systems. The CT serovars were diluted into swab transport media and a pool of processed urine specimen. Concentrations ranged from 3 Inclusion-Forming Units (IFU) per assay to 0.1 IFU per assay, which is one log below the analytical sensitivity claim for the assay of one IFU per assay (7.25 IFU/swab, 5 IFU/mL urine). Percent positivity between the Tigris DTS and DTS Systems was equivalent to 95% confidence for all three serovars down to the analytical claim level. Dilutions below the level also tested positive on both platforms. Overall, comparable sensitivity was demonstrated at a detection level of one IFU per assay between the Tigris DTS and DTS systems.

One sensitivity panel in vaginal specimen pool and one sensitivity panel in post-processed PreservCyt Solution liquid Pap specimen pool were prepared at CT 5 fg rRNA and tested 60 replicates on the Tigris DTS system. Percent positivity (95% C.I.) on the Tigris DTS system for vaginal swab specimen was 100% (95.1–100) and post-processed PreservCyt Solution liquid Pap specimen was 100% (95.1–100).

The analytical sensitivity for the Finnish variant of *Chlamydia trachomatis* (FI-nvCT) was determined by testing dilutions of *in vitro* transcript in negative urine specimens, negative ThinPrep specimens, and simulated swab matrix specimens. Thirty replicates of each dilution were tested on the Tigris DTS system with each of three reagent lots of the updated version of the Aptima Combo 2 assay for a total of 90 replicates per specimen type. The analytical sensitivity was determined to be less than one IFU per assay in urine, ThinPrep, and simulated swab matrix specimens. The detection capabilities of the updated version of the Aptima Combo 2 assay were confirmed across multiple CT variants.

Dilutions of three GC clinical isolates were tested on three Tigris DTS system and in parallel on the DTS systems. The GC isolates were diluted into swab transport media and a pool of processed urine specimen. Concentrations ranged from 150 cells per assay to 5 cells per assay, which is one log below the analytical sensitivity claim for the assay of 50 cells/assay (362 cells/swab, 250 cells/mL urine). Percent positivity between the Tigris DTS and DTS systems was equivalent to 95% confidence for all three isolates down to the analytical claim level. Dilutions below the level also tested positive on both platforms. Overall, comparable sensitivity was demonstrated at a detection level of 50 cells per assay between the Tigris DTS and DTS systems.

One sensitivity panel in vaginal specimen pool and one sensitivity panel in post-processed PreservCyt Solution liquid Pap specimen pool were prepared at GC 250 fg rRNA and tested 60 replicates on the Tigris DTS system. Percent positivity (95% C.I.) on the Tigris DTS system for vaginal swab specimen was 100% (95.1–100) and post-processed PreservCyt Solution liquid Pap specimen was 100% (95.1–100).

CT/GC rRNA Spiked Clinical Panel Study — Vaginal Swab and PreservCyt Solution Liquid Pap Specimens

The CT/GC rRNA spiked clinical panel study evaluated agreement between the two systems using two Hologic-prepared CT/GC clinical panels spiked with 0 to 5,000 fg rRNA/assay of CT and/or 0 to 250,000 fg rRNA/assay of GC. The CT/GC clinical panels were created from vaginal swab and PreservCyt Solution liquid Pap specimens collected from 309 female subjects whose specimens had negative Aptima Combo 2 Assay results on the DTS systems when tested at Hologic. Negative specimens were pooled by specimen type, spiked or not spiked with CT and/or GC rRNA, and aliquotted as replicates of each panel member. Replicates of each of 13 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.

One vaginal swab replicate from the very low CT concentration panel member (0.05 fg rRNA/assay) had an equivocal CT result on the DTS systems.

Table 25 shows the percent agreements for each level of rRNA in the vaginal swab and PreservCyt Solution liquid Pap panels, respectively, with expected CT and GC results for the Tigris DTS system and for the DTS systems. The concentrations ranged from 1 log below to 3 logs above the 5 fg rRNA/assay for CT and 250 fg rRNA/assay for GC. Also shown in Table 25 are the overall percent agreements (99.2% for the vaginal swab panel and 100% for the PreservCyt Solution liquid Pap panel).

Table 25: CT/GC rRNA Spiked Clinical Panel Agreement Study: Agreement with Expected CT and GC Results for the Vaginal Swab Panel and PreservCyt Solution Liquid Pap Panel

Panel Member CT/GC	Concentration (fg rRNA/assay)		Replicates	Vaginal Swab Panel				PreservCyt Solution Liquid Pap Panel			
				CT		GC		CT		GC	
	CT	GC		Tigris %Agrmt	DTS %Agrmt	Tigris %Agrmt	DTS %Agrmt	Tigris %Agrmt	DTS %Agrmt	Tigris %Agrmt	DTS %Agrmt
Low/Low	5	250	10	100	100	100	100	100	100	100	100
Low/High	5	250,000	10	100	100	100	100	100	100	100	100
High/Low	5000	250	10	100	100	100	100	100	100	100	100
High/High	5000	250,000	10	100	100	100	100	100	100	100	100
Very Low/Neg	0.5	0	10	100	88.9[1]	100	100	100	100	100	100
Low/Neg	5	0	10	100	100	100	100	100	100	100	100
Medium/Neg	50	0	10	100	100	100	100	100	100	100	100
High/Neg	5000	0	10	100	100	100	100	100	100	100	100
Neg/Very Low	0	25	10	100	100	100	100	100	100	100	100
Neg/Low	0	250	10	100	100	100	100	100	100	100	100
Neg/Medium	0	2500	10	100	100	100	100	100	100	100	100
Neg/High	0	250,000	10	100	100	100	100	100	100	100	100
Neg/Neg	0	0	12	100	100	100	100	100	100	100	100
				Overall Percent Agreement between Tigris and DTS (95% CI): 99.2% (95.8–100)				Overall Percent Agreement between Tigris and DTS (95% CI): 100% (97.2–100)			

DTS % Agrmt = Agreement between DTS and expected results, Tigris % Agrmt = Agreement between Tigris DTS and expected results.

[1] 1/10 replicates had equivocal CT results on the DTS systems and was excluded from this analysis. 8/9 agreed with expected results. 1/9 was CT- on the DTS systems. The CT concentration of this panel member is 1 log below 5 fg rRNA/assay.

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 Combo 2 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 15, including 3 organisms that are most closely related to CT and 17 organisms that are most closely related to GC. All of the organisms tested produced negative results on the Tigris DTS system.

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 Tigris DTS and equivalence between the Tigris DTS system and DTS systems with respect to this potential interferant. Fresh blood was added to clinical swab, vaginal swab, post-processed PreservCyt Solution liquid Pap, and urine specimen pools, then tested for potential assay interference in the absence and presence of CT and GC target. The estimated rRNA equivalent of one CT IFU/assay (5 fg/assay) and 50 GC cells/assay (250 fg/assay) were used as these represent the analytical sensitivity of the 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% (vol/vol) blood in swab specimens, vaginal swab specimens, post-processed PreservCyt Solution liquid Pap specimens and 30% (vol/vol) blood in urine specimens. All samples that did not contain target were correctly identified as negative for both CT and GC. These results are identical to those demonstrated for the DTS systems when spiked with the same quantities of blood.

Blood added to swab, vaginal swab, post-processed PreservCyt Solution liquid Pap specimens, and urine specimens at levels much higher than could be expected with normal specimen collection, did not interfere with results 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 multi-day analytical study was conducted using spiked panels on three Tigris DTS system. The study used 20% high-target GC samples containing 1.0×10^9 cells/reaction, which were randomly spaced amongst 80% negative samples containing swab transport media. Over the course of the study, 1,372 high-target samples and 5,516 negative samples were tested across the three Tigris DTS system. The overall carryover rate, including both false positive and equivocal results, averaged 0.3% (18/5491). A total of 25 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, including both false positive and equivocal results, averaged 1.1% (12/1097). For false positives in this subset, the carryover rate ranged from 0% to 1.1% across the three Tigris DTS system. For equivocals in this subset, the carryover rate ranged from 0% to 0.9% across the three Tigris DTS system. These results demonstrate that carryover contamination is minimized on the Tigris DTS system.

Panther System Analytical Performance

Spiked Clinical Panel Agreement Study

Individual negative urine specimens were spiked with CT serovar G, GC, or a combination of CT and GC to create a panel of 120 CT positives, 120 GC positives, and 120 dual positive panel members. 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). GC positive panel members were spiked with organisms at 12.5 CFU/mL, 125 CFU/mL, or 1,250 CFU/mL (25 fg/assay, 250 fg/assay, or 2,500 fg/assay). Dual positives were spiked with CT organisms at 2.5 IFU/mL (5 fg/assay) and GC organisms at 2,500,000 CFU/mL (5,000,000 fg/assay) or CT at 25 IFU/mL (50 fg/assay) and GC at 1,250 CFU/mL (2,500 fg/assay) or CT at 25,000 IFU/mL (50,000 fg/assay) and GC at 125 CFU/mL (250 fg/assay) or CT at 2.5 IFU/mL (5 fg/assay) and GC at 125 CFU/mL (250 fg/assay). In addition, 120 CT and GC negative urine specimens were collected. The positive and negative panels were tested on three Panther systems 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 99.5 for CT and GC. Negative percent agreement between the Panther systems and the Tigris DTS systems was 99.9% with a lower 95% confidence interval of 99.5. The results of the study are shown in Table 26.

Table 26: Spiked Clinical Panel Agreement Study: Agreement with Expected CT and GC Results

Panel Member	Concentration (IFU or CFU/mL)		Concentration (fg/assay)		Replicates	CT		GC	
	CT	GC	CT	GC		Tigris %Agrmt	Panther %Agrmt	Tigris %Agrmt	Panther %Agrmt
CT/GC Panels[1,2]									
Low/Low	2.5	125	5	250	90	100	100	100	100
Medium/Medium	25	1,250	50	2,500	90	100	100	100	100
Low/High	2.5	2,500,000	5	5,000,000	90	100	100	100	100
High/Low	25,000	125	50,000	250	90	100	100	100	100
GC Panels[2,3]									
Neg/Very Low	0	12.5	0	25	117*	100	100	100	100
Neg/Low	0	125	0	250	120	100	100	100	100
Neg/Medium	0	1,250	0	2,500	120	100	99.2	100	100
CT Panels[1,3]									
Very Low/Neg	0.25	0	0.5	0	120	100	100	100	100
Low/Neg	2.5	0	5	0	120	100	100	100	100
Medium/Neg	25	0	50	0	120	100	100	100	100
Negative Panels[3]									
Neg/Neg	0	0	0	0	360	100	100	99.7	99.7

*One panel member was manufactured incorrectly and was excluded from the analysis.

[1]Overall CT Positive Percent Agreement between Tigris and Panther (95% CI): 100% (99.5–100).

[2]Overall GC Positive Percent Agreement between Tigris and Panther (95% CI): 100% (99.5–100).

[3]Overall Negative Percent Agreement between Tigris and Panther (95% CI): 99.9% (99.5–100).

The clinical panel agreement study evaluated the equivalence between the original and updated versions of the Aptima Combo 2 assay using 20 prepared CT/GC clinical panels containing 0 to 2,500 IFU/mL of wild type CT, 0 to 500 IFU/mL of FI-nvCT, and 0 to 125,000 CFU/mL of GC in urine specimens. Each of the 20 panels were tested in triplicate in two

runs per day on three Panther systems by two operators using three lots of reagents over six days. Table 27 shows the percent agreements with expected CT and GC results for the two versions of the Aptima Combo 2 assay.

Table 27: Original and Updated Version Aptima Combo 2 Assay CT/GC Clinical Panel Agreement Study

Panel Member Concentration			CT				GC			
CT IFU/mL	FI-nvCT IFU/mL*	GC CFU/mL	Original AC2 Expected Result	Original AC2% Agreement	Updated AC2 Expected Result	Updated AC2% Agreement	Original AC2 Expected Result	Original AC2% Agreement	Updated AC2 Expected Result	Updated AC2% Agreement
0	0	0	Neg	100%	Neg	100%	Neg	100%	Neg	100%
0	0	12.5	Neg	100%	Neg	100%	Pos	100%	Pos	100%
0	0	125	Neg	100%	Neg	100%	Pos	100%	Pos	100%
0	0	1,250	Neg	100%	Neg	100%	Pos	100%	Pos	100%
0	0	125,000	Neg	100%	Neg	100%	Pos	100%	Pos	100%
0.25	0	0	Pos	100%	Pos	100%	Neg	100%	Neg	100%
2.5	0	0	Pos	100%	Pos	100%	Neg	100%	Neg	100%
25	0	0	Pos	100%	Pos	100%	Neg	100%	Neg	100%
2,500	0	0	Pos	100%	Pos	100%	Neg	100%	Neg	100%
0	0.02	0	Neg	100%	Pos	100%	Neg	100%	Neg	100%
0	0.05	0	Neg	100%	Pos	100%	Neg	100%	Neg	100%
0	0.2	0	Neg	98.2%	Pos	100%	Neg	99.1%	Neg	100%
0	500	0	Neg	100%	Pos	100%	Neg	100%	Neg	100%
2.5	0	125	Pos	100%	Pos	100%	Pos	100%	Pos	100%
25	0	1,250	Pos	100%	Pos	100%	Pos	100%	Pos	100%
2,500	0	125	Pos	100%	Pos	100%	Pos	100%	Pos	100%
2.5	0	125,000	Pos	100%	Pos	100%	Pos	100%	Pos	100%
0	500	125	Neg	100%	Pos	100%	Pos	100%	Pos	100%
0	0.05	125,000	Neg	100%	Pos	100%	Pos	100%	Pos	100%
2,500	500	125	Pos	100%	Pos	100%	Pos	100%	Pos	100%

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

Analytical Sensitivity Study

Analytical sensitivity of the Aptima Combo 2 assay was tested using three representative sample matrices. These were urine processed with Urine Transport Medium (UTM), PreservCyt Liquid Pap Solution liquid diluted with Swab Transport Medium (STM), and STM. CT and GC rRNA were spiked into pools of these three matrices at the following concentrations at RNA equivalent 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) for CT or 25 fg/assay, 250 fg/assay, or 2500 fg/assay for GC (rRNA equivalents of 12.5 CFU/mL, 125 CFU/mL or 1,250 CFU/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 three lots of reagents in replicates of 96. Agreement with the expected result was calculated. Agreement to expected results was 100% (95% CI 96.1–100%) for all urine panels, 100% (95% CI 96.0–100%) for all PreservCyt Solution liquid Pap solution panels, and 100% (95% CI 96.1–100%) for all STM panels. The analytical sensitivity for the assay is 2.5 IFU/mL for CT and 125 CFU/mL for GC.

The analytical sensitivity for FI-nvCT was determined by testing dilutions of *in vitro* transcript in negative urine specimens, negative ThinPrep specimens, and simulated swab matrix specimens. Thirty replicates of each dilution were tested on the Panther system with each of three reagent lots of the updated version of the Aptima Combo 2 assay for a total of 90 replicates per specimen type. The analytical sensitivity was determined to be less than one IFU per assay in urine, ThinPrep, and simulated swab matrix specimens. The detection capabilities of the updated version of the Aptima Combo 2 assay were confirmed across multiple CT variants.

Reproducibility Study

The Aptima Combo 2 assay precision was evaluated across three Panther systems and three Aptima Combo 2 assay kit lots over a period of 24 days. Panels were made by spiking CT and/or GC rRNA into STM at the concentrations shown in Table 28. 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 (37). The total number of replicates for each panel was 96. Table 28 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, as well as total variability.

Table 28: Panther Precision for Aptima Combo 2 Assay

Matrix	CT (IFU/mL)	GC (CFU/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	0	96	6	100	0.06	1	0.88	13.5	0	0	1.02	15.7	1.3	20.1
	0.25	0	95	1226	100	70.03	5.7	20.03	1.6	8.43	0.7	47.05	3.8	87.1	7.1
	2.5	0	96	1249	100	77.97	6.2	6.11	0.5	0	0	32.87	2.6	84.8	6.8
	25	0	95	1268	100	72.85	5.7	15.3	1.2	0	0	39.58	3.1	84.3	6.6
	0	12.5	96	1081	100	18.44	1.7	28.59	2.6	0	0	26.68	2.5	43.2	4
	0	125	96	1266	100	29.81	2.4	0	0	8.86	0.7	27.58	2.2	41.6	3.3
	0	1250	96	1309	100	29.41	2.2	0	0	9.83	0.8	31.83	2.4	44.4	3.4
	2.5	125	96	2456	100	86.58	3.5	0	0	0	0	52.99	2.2	101.5	4.1
	2.5	2500	96	2509	100	73.13	2.9	0	0	19.8	0.8	46.77	1.9	89	3.5
	1000	2500	96	2496	100	31.72	1.3	6.14	0.2	0	0	193.66	7.8	196.3	7.9
	1000	125	96	2471	100	83.63	3.4	9.36	0.4	0	0	52.35	2.1	99.1	4
Urine	0	0	94	6	100	0.2	3.2	0.66	10.8	0.36	5.9	1	16.3	1.3	21.2
	0.25	0	95	863	100	70.73	8.2	165.65	19.2	47.97	5.6	132.27	15.3	228.6	26.5
	2.5	0	95	1129	100	56.02	5	89.56	7.9	8.56	0.8	74.19	6.6	129.4	11.5
	25	0	96	1246	100	60.45	4.9	13.97	1.1	13.36	1.1	43.03	3.5	76.7	6.2
	0	12.5	96	1016	100	18.83	1.9	31.81	3.1	7.88	0.8	49.53	4.9	62.3	6.1
	0	125	96	1209	100	49.32	4.1	23.5	1.9	1.68	0.1	40.28	3.3	67.9	5.6
	0	1250	96	1252	100	53.01	4.2	40.34	3.2	7.72	0.6	40.23	3.2	78.2	6.2
	2.5	125	95	2290	100	73.92	3.2	40.88	1.8	10.43	0.5	56.12	2.5	101.9	4.4
PreservCyt	0	0	96	7	100	0	0	0.8	11.7	0	0	1.54	22.4	1.7	24.7
	0.25	0	96	1113	100	92.29	8.3	30.08	2.7	0	0	63.57	5.7	116	10.4
	2.5	0	96	1194	100	62.54	5.2	24.83	2.1	0	0	47.01	3.9	82.1	6.9
	25	0	95	1222	100	65.14	5.3	26.36	2.2	14.67	1.2	34.97	2.9	79.8	6.5
	0	12.5	93	994	100	33.28	3.3	36.92	3.7	15.97	1.6	26.15	2.6	58.4	5.9
	0	125	95	1189	100	40.1	3.4	4.45	0.4	10.87	0.9	21.44	1.8	47	4
	0	1250	95	1239	100	37.69	3	7.47	0.6	13.61	1.1	18.04	1.5	44.6	3.6
	2.5	125	95	2333	100	99.68	4.3	35.27	1.5	12.61	0.5	48.86	2.1	117.2	5

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 Study

The analytical specificity of the updated version of the Aptima Combo 2 assay was evaluated using a subset of microorganisms listed in Table 15 and Table 16. The 86 microorganisms tested consisted primarily of viral, bacterial, and yeast strains. None of the microorganisms tested were found to have an impact on the performance or analytical specificity of the updated version of the Aptima Combo 2 assay.

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 and GC target. The estimated rRNA equivalents of one CT IFU/assay (5 fg/assay) and 50 GC cells/assay (250 fg/assay) were used as target concentrations as these represent 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 for both CT and GC. 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, 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 GC 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 GC rRNA spiked into STM to give a final concentration of 5 x 10^5 fg rRNA/reaction (rRNA equivalent of 2.5 x 10^5 CFU/mL). Testing was carried out using 5 runs on each of three Panther systems with a total of 2,936 negative samples. The overall carryover rate was 0% with a 95% confidence interval of 0–0.1%. Four negative samples were reported as invalid and were excluded from the calculation.

Clinical Specimen Agreement Study

The clinical specimen agreement between the original version and updated version of the Aptima Combo 2 assay was evaluated using remnant swab specimens collected from patients undergoing *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) screening. A single replicate of each specimen was tested with both the original version and the updated version of the Aptima Combo 2 assay on the Panther system. Table 29 and Table 30 show the CT and GC positive, negative, and overall percent agreement for the 325 specimens evaluated.

Table 29: Chlamydia trachomatis Clinical Specimen Agreement Study

		Original Version AC2 Assay	
		CT Positive	**CT Negative**
Updated Version AC2 Assay	**CT Positive**	49	3
	CT Negative	0	273

Positive Percent Agreement (95% C.I.): 100% (92.7% - 100%)
Negative Percent Agreement (95% C.I.): 98.9% (96.9% - 99.6%)
Overall Percent Agreement (95% C.I.): 99.1% (97.3% - 99.7%)

Table 30: Neisseria gonorrhoeae Clinical Specimen Agreement Study

		Original Version AC2 Assay	
		GC Positive	**GC Negative**
Updated Version AC2 Assay	**GC Positive**	47	1
	GC Negative	0	275

Positive Percent Agreement (95% C.I.): 100% (92.4% - 100%)
Negative Percent Agreement (95% C.I.): 99.6% (98.0% - 99.9%)
Overall Percent Agreement (95% C.I.): 99.7% (98.3% - 99.9%)

Two samples with GC equivocal results were excluded from this analysis.

Extragenital Specimen Types (Throat and Rectal Swab Specimens)

Summary

Collectively, the analytical and clinical data provided below support the use of the Aptima Combo 2 Assay for testing throat and rectal swab specimens for the qualitative detection and differentiation of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) to aid in the diagnosis of chlamydial and/or gonococcal disease.

Analytical Sensitivity Study

The 95% limit of detection for the extragenital swabs with the Aptima Combo 2 Assay was determined for throat and rectal swabs. Two CT Serovars (E and G) and two clinical GC isolates were spiked into pools of these swabs. The panels were tested on two Panther systems using one reagent lot in replicates of at least 20 over eight days.

The 95% limit of detection for throat swabs is 0.005 IFU/ml (95% CI 0.003-0.020) for CT and 0.10 CFU/ml (95% CI 0.09-0.13) for GC. The 95% limit of detection for rectal swabs is 0.007 IFU/ml (95% CI 0.005-0.023) for CT and 0.10 CFU/ml (95% CI 0.09-0.12) for GC.

Clinical Performance Data

Clinical performance data was evaluated from 15 articles of scientific literature (1, 2, 3, 13, 16, 19, 21, 31, 34, 38, 39, 48, 49, 52, 53) each of which reported use of the Aptima Combo 2 Assay in testing extragenital specimens.

For CT throat swab specimens, the studies reported point estimates of 100% for sensitivity and 100% for specificity (38). For CT rectal swab specimens, the studies reported sensitivity point estimates ranging from 71% to 100% and specificity point estimates from 95.6% to 100% (1, 2, 3, 13, 34, 38).

For GC throat swab specimens, the studies reported sensitivity point estimates ranging from 88.2% to 100% and specificity point estimates from 87.8% to 100% (2, 38). For GC rectal swab specimens, the studies reported sensitivity point estimates ranging from 75% to 100% and specificity point estimates ranging from 87.9% to 100% (3, 13, 21, 34, 38, 48).

Cross-Reactivity of Microorganisms

For a list of microorganisms tested for cross-reactivity in throat and rectal swabs, refer to Table 16.

Potential Interfering Substances

The following interfering substances that may be found on extragenital swabs were individually spiked into STM: cold sore medication, lip balm, hemorrhoidal cream, human feces, cough suppressant, toothpaste, mouthwash, laxative suppository, anti-diarrheal medication, and antacid. All were tested for potential assay interference in the absence and presence of CT and GC at 3X the 95% limit of detection of the sample type. Samples spiked with CT and GC showed at least 95% positivity in the presence of the substances. Substances not spiked with CT or GC did not give a positive result for either CT or GC.

Sample Handling and Stability

Data to support the recommended storage conditions for extragenital swab samples were generated with pooled negative swab samples. Throat and rectal pools were spiked with CT and GC at concentrations of 2X the 95% limit of detection per each swab sample type. The spiked samples were held at -70°C, -20°C, 4°C, and 30°C. Samples were tested at days 0, 8, 15, 23, 36, and 60. All test conditions were at least 95% positive for both CT and GC at all times and temperatures.

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