Aptima Combo 2® Assay (Panther® System)

For *in vitro* diagnostic use.

Rx 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 urogenital disease using the Panther® System as specified.

On the Panther System, the assay may be used to test the following specimens from symptomatic and asymptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens, clinician-collected gynecological specimens collected in the PreservCyt® Solution, patient-collected vaginal swab specimens,[1] and female and male urine specimens.

[1]Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The vaginal and multitest swab specimen collection kits are 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, an estimated 1,598,354 (497 cases per 100,000 population) new cases of CT and 468,514 (146 per 100,000 population) new cases of GC infections were reported to the Centers for Disease Control in 2016 (6).

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 (31). The serovars D through K are the major cause of genital chlamydial infections in men and women (23). *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and Pelvic Inflammatory Disease (PID) (3, 16, 25, 26). *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, 12, 24).

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 (2, 10, 18, 27). Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe assays 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, which can manifest as endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscesses. A smaller percentage of persons with gonococcal infections may develop Disseminated Gonococcal Infection (DGI) (15, 21).

Conventional diagnosis of GC infection requires isolation of the organism on selective media or the observation of diplococci in Gram stained smears (17). 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. Poor sampling technique, toxic sampling materials, and the inhibition of growth by components of body secretions can also result in false negative results (8, 19). Commonly used 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 (7, 11, 14, 20, 22, 28, 29, 30). 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 (9, 13). The Aptima Combo 2 Assay on the Panther System qualitatively detects CT and/or GC rRNA in clinician-collected endocervical, vaginal, and male urethral swab specimens, patient-collected vaginal swab specimens, PreservCyt Solution liquid Pap specimens, and female and male urine specimens from symptomatic and asymptomatic individuals.

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

Laboratory Related

C. Use only supplied or specified disposable laboratory ware.

D. 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.

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

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

Specimen Related

G. This assay has been cleared for the following specimens on the Panther System:

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

Only specimens collected with the following specimen collection kits have been cleared on the Panther System:

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

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

H. 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.

I. 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 the Aptima Combo 2 Assay.

J. 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.

K. 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.

L. 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.

M. 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.

N. 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.

O. 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.

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

Assay Related

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

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

S. Some reagents of this kit are labeled with risk and safety symbols.

Note: *For hazard communication information, refer to the Safety Data Sheet Library at www.hologicsds.com.*

US Hazard Information	
	**Selection Reagent** ***BORIC ACID 1-5%*** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation P264 - Wash face, hands and any exposed skin thoroughly after handling P280 - Wear protective gloves/protective clothing/eye protection/face protection P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P337 + P313 - If eye irritation persists: Get medical advice/attention P302 + P352 - IF ON SKIN: Wash with plenty of soap and water P332 + P313 - If skin irritation occurs: Get medical advice/attention P362 - Take off contaminated clothing and wash before reuse

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, whichever comes first.

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

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

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

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

K. Do not freeze the reagents.

Specimen Collection and Storage

The Aptima Combo 2 Assay is designed to detect the presence of CT and GC in the following specimens: endocervical and male urethral swab specimens, vaginal swab specimens, PreservCyt Solution liquid Pap specimens, and female and male urine specimens.

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 116 days 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 or ThinPrep 3000 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. Transfer 1 mL of the fluid remaining in the PreservCyt Solution vial into an Aptima Specimen Transfer tube according to the instructions in the Aptima Specimen Transfer Kit package insert.

d. Once the PreservCyt Solution liquid Pap specimen is transferred to the Aptima Specimen Transfer tube, the specimen must be assayed with the Aptima 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.*

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. 302923)

250 tests (2 boxes and 1 Controls kit) (Cat. No. 303094)

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 2 Target Capture Reagent B** *Non-infectious nucleic acid 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) (Continued)
(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 Vaginal Swab Specimen Collection Kit	301162

	Cat. No.
Aptima Multitest Swab Specimen Collection Kit[1]	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% (0.7M to 1.0M) 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)*

[1] For Aptima Combo 2 Assay, the Aptima Multitest Swab Specimen Collection Kit has been validated for the collection of vaginal swab specimens.

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

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) 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 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the 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 bottle to mix. Avoid creating foam while swirling the bottle (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).

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

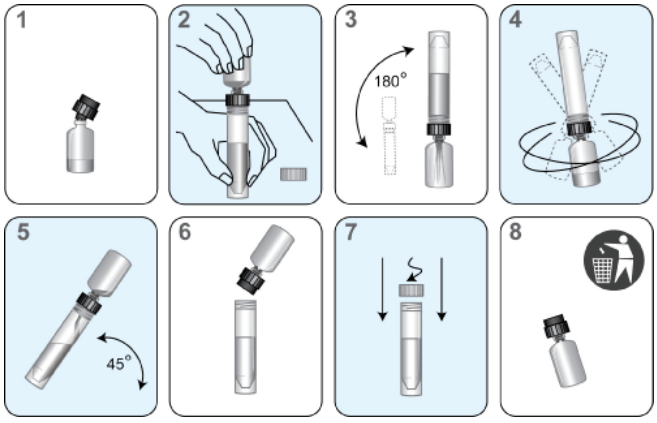

Figure 1. Panther System Reconstitution Process

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

E. System Preparation
   1. Set up the system according to the instructions in the *Panther System Operator's Manual* and *Procedural Notes*. Make sure that the appropriately sized reagent racks and TCR adapters are used.
   2. Load samples.

Procedural Notes

A. Controls
   1. To work properly with the Aptima Assay software for the Panther System, one pair of controls is required. The Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control CT tubes can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Patient specimen pipetting will begin when one of the following two conditions has been met:
      a. A pair of controls is currently being processed by the system.
      b. Valid results for the controls are registered on the system.
   2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated 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 specimen transport medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

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 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. Negative controls may not be effective in monitoring random carryover. See *Panther System Analytical Performance* for results from a high-target analytical carryover study that was performed to demonstrate control of carryover on the Panther System.

C. Specimen Preparation Control (Optional)

The 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*.

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.
- Testing of an endocervical specimen is recommended for female patients who are clinically suspected of having a chlamydial or gonococcal infection. If both a Pap and endocervical swab are collected, the PreservCyt Solution liquid Pap specimen must be collected before the endocervical swab specimen.

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

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

C. 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.

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

E. The Aptima 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 (4).

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

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

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

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

J. The Aptima 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.

K. 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.

L. PreservCyt Solution liquid Pap specimens processed with instruments other than the ThinPrep 2000 processor have not been evaluated for use in Aptima Assays.

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

N. The patient-collected vaginal swab specimen application is limited to health care facilities where support/counseling is available to explain procedures and precautions.

O. The Aptima Combo 2 Assay has not been validated for use with vaginal swab specimens collected by patients at home.

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

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

R. 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 Specimen Transport Medium, 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.

S. Customers must independently validate an LIS transfer process.

T. First catch female urine specimens are acceptable but may detect up to 10% fewer CT/GC infections when compared with vaginal and endocervical swab specimens (5).

Panther System Expected Values

Prevalence

The prevalence of CT and GC in patient populations depends on risk factors such as age, gender, the presence or absence of symptoms, the type of clinic, and the sensitivity of the test used to detect infections. A summary of the positivity of three CT and GC disease outcomes, as determined by the Aptima Combo 2 Assay on the Panther System, is shown in Tables 1, 2, and 3 for three multi-center clinical studies by clinical site and overall.

Table 1: Clinical Study 1. Positivity of CT and GC Infections as Determined by the Aptima Combo 2 Assay in Male Urethral Swab, Vaginal Swab, PreservCyt Solution Liquid Pap, and Endocervical Swab Samples by Clinical Site

Site	Positivity % (# positive/# tested with valid results)											
	MS			CVS/PVS			PCyt			FS		
	CT+/GC-	CT-/GC+	CT+/GC+	CT+/GC-	CT-/GC+	CT+/GC+	CT+/GC-	CT-/GC+	CT+/GC+	CT+/GC-	CT-/GC+	CT+/GC+
1	0 (-)	0 (-)	0 (-)	9.9 (21/212)	3.3 (7/212)	3.8 (8/212)	8.9 (20/225)	2.7 (6/225)	3.1 (7/225)	10.4 (20/193)	3.1 (6/193)	3.6 (7/193)
2	13.9 (28/202)	5.9 (12/202)	3.0 (6/202)	8.3 (19/230)	3.9 (9/230)	1.3 (3/230)	8.8 (21/239)	4.6 (11/239)	0.8 (2/239)	8.2 (19/231)	4.8 (11/231)	0.9 (2/231)
3	1.3 (1/76)	1.3 (1/76)	0.0 (0/76)	2.7 (6/222)	0.5 (1/222)	0.0 (0/222)	3.1 (7/226)	0.4 (1/226)	0.0 (0/226)	2.7 (6/223)	0.4 (1/223)	0.0 (0/223)
4	24.4 (33/135)	1.5 (2/135)	4.4 (6/135)	11.7 (40/342)	1.5 (5/342)	1.2 (4/342)	10.2 (35/342)	1.5 (5/342)	0.9 (3/342)	11.3 (38/337)	1.8 (6/337)	0.9 (3/337)
5	0 (-)	0 (-)	0 (-)	4.5 (1/22)	0.0 (0/22)	0.0 (0/22)	4.8 (1/21)	0.0 (0/21)	0.0 (0/21)	4.3 (1/23)	0.0 (0/23)	0.0 (0/23)
6	21.5 (28/130)	5.4 (7/130)	0.8 (1/130)	11.9 (13/109)	3.7 (4/109)	0.9 (1/109)	8.7 (10/115)	1.7 (2/115)	0.9 (1/115)	8.8 (10/114)	1.8 (2/114)	0.9 (1/114)
7	16.7 (1/6)	0.0 (0/6)	0.0 (0/6)	3.2 (5/157)	2.5 (4/157)	0.6 (1/157)	2.5 (4/161)	2.5 (4/161)	0.6 (1/161)	2.6 (4/152)	2.6 (4/152)	0.7 (1/152)
All	16.6 (91/549)	4.0 (22/549)	2.4 (13/549)	8.1 (105/1294)	2.3 (30/1294)	1.3 (17/1294)	7.4 (98/1329)	2.2 (29/1329)	1.1 (14/1329)	7.7 (98/1273)	2.4 (30/1273)	1.1 (14/1273)

CVS = clinician-collected vaginal swab, FS = female endocervical swab, MS = male urethral swab, PCyt = PreservCyt Solution liquid Pap, PVS = patient-collected vaginal swab.

Table 2: Clinical Study 1 and Clinical Study 2. Positivity of CT and GC Infections as Determined by the Aptima Combo 2 Assay in Male Urine Samples by Clinical Site

Site	Positivity % (# positive/# tested with valid results)		
	CT+/GC-	CT-/GC+	CT+/GC+
1	6.0 (6/100)	0.0 (0/100)	0.0 (0/100)
2	3.0 (2/67)	3.0 (2/67)	0.0 (0/67)
3	0.0 (0/109)	0.9 (1/109)	0.0 (0/109)
4	13.0 (13/100)	3.0 (3/100)	1.0 (1/100)
5	13.6 (17/125)	5.6 (7/125)	0.0 (0/125)
6	15.1 (43/284)	7.0 (20/284)	2.1 (6/284)
7	1.4 (3/212)	0.9 (2/212)	0.0 (0/212)
8	1.3 (1/75)	0.0 (0/75)	0.0 (0/75)
9	16.7 (42/251)	5.2 (13/251)	3.2 (8/251)
10	20.5 (17/83)	1.2 (1/83)	0.0 (0/83)
11	4.1 (6/146)	0.7 (1/146)	0.7 (1/146)
12	14.3 (16/112)	4.5 (5/112)	2.7 (3/112)
13	8.9 (10/112)	2.7 (3/112)	2.7 (3/112)
14	7.7 (2/26)	0.0 (0/26)	0.0 (0/26)
All	9.9 (178/1802)	3.2 (58/1802)	1.2 (22/1802)

Note. CT and GC prevalence was estimated using symptomatic male urine samples from Clinical Study 2 and asymptomatic male urine samples from both studies.

Table 3: Clinical Study 3. Positivity of CT and GC Infections as Determined by the Aptima Combo 2 Assay in Female Urine Samples by Clinical Site

Site	Positivity % (# positive/# tested with valid results)		
	CT+/GC-	CT-/GC+	CT+/GC+
1	14.8 (23/155)	3.2 (5/155)	1.9 (3/155)
2	2.5 (5/199)	0.0 (0/199)	0.0 (0/199)
3	2.0 (4/199)	0.0 (0/199)	0.0 (0/199)
4	6.3 (5/79)	0.0 (0/79)	0.0 (0/79)
5	5.1 (5/99)	0.0 (0/99)	0.0 (0/99)
6	9.8 (15/153)	2.0 (3/153)	2.0 (3/153)
7	7.3 (18/247)	0.0 (0/247)	0.0 (0/247)
8	7.4 (14/189)	1.1 (2/189)	0.0 (0/189)
9	6.7 (6/90)	0.0 (0/90)	1.1 (1/90)
10	6.1 (6/99)	0.0 (0/99)	0.0 (0/99)
11	3.2 (3/93)	0.0 (0/93)	0.0 (0/93)
12	0.0 (0/97)	0.0 (0/97)	0.0 (0/97)
13	8.7 (26/299)	1.0 (3/299)	0.3 (1/299)
14	4.6 (9/196)	0.0 (0/196)	0.0 (0/196)
15	5.0 (5/100)	0.0 (0/100)	0.0 (0/100)
16	8.8 (23/261)	1.5 (4/261)	0.8 (2/261)
17	20.0 (5/25)	4.0 (1/25)	0.0 (0/25)
All	6.7 (172/2580)	0.7 (18/2580)	0.4 (10/2580)

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive and negative predictive values (PPV and NPV) of the Aptima Combo 2 Assay for different hypothetical prevalence rates are shown for each specimen type in Table 4. For each specimen type, the PPV and NPV are derived for different hypothetical prevalence rates using the sensitivity and specificity estimates from two multi-center clinical studies (see Tables 5 and 9).

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

Specimen Type	Hypothetical Prevalence (%)	CT Detection		GC Detection	
		PPV (%)	NPV (%)	PPV (%)	NPV (%)
Clinician-Collected Vaginal Swab/Patient-Collected Vaginal Swab	1	38.9	100	70.6	100
	2	56.3	99.9	82.9	100
	5	76.8	99.9	92.6	99.9
	10	87.5	99.7	96.3	99.7
	15	91.7	99.5	97.7	99.6
	20	94.0	99.3	98.3	99.4
	25	95.5	99.1	98.8	99.2
PreservCyt Solution Liquid Pap	1	100	100	100	100
	2	100	100	100	100
	5	100	99.9	100	100
	10	100	99.8	100	100
	15	100	99.7	100	100
	20	100	99.6	100	100
	25	100	99.4	100	100
Female Endocervical Swab	1	58.5	100	85.8	100
	2	74.0	99.9	92.4	100
	5	88.0	99.9	96.9	100
	10	93.9	99.7	98.5	100
	15	96.1	99.5	99.1	100
	20	97.2	99.3	99.3	100
	25	97.9	99.1	99.5	100
Male Urethral Swab	1	53.1	100	100	100
	2	69.6	100	100	100
	5	85.5	100	100	100
	10	92.6	100	100	100
	15	95.2	100	100	100
	20	96.6	100	100	100
	25	97.4	100	100	100
Male Urine	1	83.6	100	77.4	100
	2	91.2	99.9	87.4	100
	5	96.4	99.7	94.7	99.9
	10	98.2	99.5	97.4	99.9
	15	98.9	99.2	98.4	99.8
	20	99.2	98.8	98.8	99.7
	25	99.4	98.4	99.1	99.6

Note. Aptima Combo 2 Assay performance was estimated using vaginal swab, PreservCyt Solution liquid Pap, female endocervical swab, and male urethral swab sample results from Clinical Study 1, symptomatic male urine samples from Clinical Study 2, and asymptomatic male urine samples from Clinical Studies 1 and 2.

Panther System Clinical Performance

Three clinical studies were performed. Aptima Combo 2 Assay clinical performance was estimated with male urethral swab, vaginal swab, PreservCyt Solution liquid Pap, and endocervical swab specimens in Clinical Study 1, with male urine specimens in Clinical Study 2, and with female urine specimens in Clinical Study 3.

Clinical Study 1. Vaginal Swab, PreservCyt Solution Liquid Pap, Female Endocervical Swab, and Male Urethral Swab Specimen Clinical Study[2]

A prospective, multi-center clinical study was conducted to establish the performance characteristics of the Aptima Combo 2 Assay on the Panther System. Specimens were collected from symptomatic and asymptomatic men (n=580) and women (n=1332) enrolled from 7 geographically and ethnically diverse US clinical sites, including obstetrics and gynecology, family planning, public health, and STD clinics. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. Of the 580 male subjects, none were <18 years of age, 72 were 18 to 20 years of age, 201 were 21 to 25 years of age, and 307 were >25 years of age. Of the 1332 female subjects, 11 were 14 to 15 years of age, 59 were 16 to 17 years of age, 319 were 18 to 20 years of age, 401 were 21 to 25 years of age, and 542 were >25 years of age.

Up to 2 specimens were collected from each male subject (1 urethral swab and 1 first-catch urine, in that order) and up to 4 specimens were collected from each female subject (1 first-catch urine, 1 vaginal swab, 1 PreservCyt Solution liquid Pap specimen, and 1 endocervical swab, in that order). All specimens were clinician-collected except urine specimens and approximately half of the vaginal swab specimens, which were collected by the subject at the clinic. Approximately half of the PreservCyt Solution liquid Pap specimens were collected with a broom-type device and half were collected with a spatula and cytobrush. Samples were prepared for Aptima testing in accordance with the appropriate Aptima specimen collection kit package insert instructions.

All evaluable samples (567 male urethral swab, 580 male urine, 1319 vaginal swab, 1330 PreservCyt Solution liquid Pap, and 1310 endocervical swab samples) were tested with the Aptima Combo 2 Assay on the Panther System in accordance with package insert instructions. The samples were split among three laboratories (two external laboratories and in-house). Samples with initial invalid, equivocal, or error results were retested. Eighteen (18) male urethral swab, 25 vaginal swab, 1 PreservCyt Solution liquid Pap, and 37 endocervical swab samples had final invalid results and were excluded from the analyses. Most of the invalid results were due to insufficient sample volume. One vaginal swab and 1 endocervical swab had final CT equivocal results and 1 PreservCyt Solution liquid Pap sample and 1 endocervical swab had final GC equivocal results and were excluded from the analyses.

Male urethral swab, male and female urine, and PreservCyt Solution liquid Pap samples were tested with cleared nucleic acid amplification tests (NAATs) to establish the infected status. The infected status algorithm used results from two specimen types and two reference NAATs. Subjects were categorized as infected if a positive result occurred in each of the two reference NAATs (see Tables 13, 14, 17, and 18 for the infected status algorithms). For female subjects, if the positive NAAT results occurred only in the urine specimens and not in the PreservCyt Solution liquid Pap specimens, the subject was categorized as infected; however, for the

2 This study included testing of male urine samples with the Aptima Combo 2 Assay on the Panther System that were not included in the original performance results due to the low prevalence of GC in the study population.

evaluation of the non-urine specimen types, the specimens were considered non-infected. Subjects that could not be categorized as infected or not infected were excluded from the performance analyses.

In addition, male urine samples tested with the Aptima Combo 2 Assay on the Panther System were excluded from the performance analyses due to the low prevalence of GC in the study population, particularly in the asymptomatic subjects.

Clinical Study 2. Male Urine Specimen Clinical Study

A prospective, multi-center clinical study was conducted to establish the performance characteristics of the Aptima Combo 2 Assay on the Panther System in male urine specimens. Specimens were collected from symptomatic and asymptomatic men (n=1492) enrolled from 13 geographically and ethnically diverse US clinical research sites, and family planning, public health, men's health, and STD clinics. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. Of the 1492 subjects enrolled, 14 were withdrawn.

Two specimens were collected from each subject (1 urethral swab and 1 first-catch urine, in that order). The urethral swab specimens were clinician-collected, and urine specimens were collected by the subject at the clinic. Urine specimens from each subject were processed into multiple samples for CT/GC testing with different NAATs in accordance with the instructions in the appropriate specimen collection kit package insert. The male urine samples for Aptima Combo 2 Assay testing on the Panther System were split among three external laboratories.

All 1478 male urine samples from non-withdrawn subjects were tested with the Aptima Combo 2 Assay on the Panther System in accordance with the Aptima Combo 2 Assay package insert instructions. Samples with initial invalid, equivocal, or error results were retested. One male urine sample had a final invalid result and was excluded from the analyses. The invalid result was due to insufficient sample volume. Of the remaining 1477 evaluable male subjects, 46 were 16 to 17 years of age, 155 were 18 to 20 years of age, 524 were 21 to 30 years of age, 279 were 31 to 40 years of age, and 473 were >40 years of age.

Male urethral swab and urine samples were tested with cleared NAATs to establish the infected status (see Tables 15 and 19 for the infected status algorithms). The infected status algorithm used urethral swab and urine sample results from one reference CT and GC NAAT and urine sample results from two additional reference CT and GC NAATs to generate four reference results for each analyte. Subjects were categorized as infected if a positive result occurred in at least two of the reference NAATs. Subjects that could not be categorized as infected or not infected were excluded from the performance analyses; 1 subject had an indeterminate CT infected status and was excluded from the performance analyses for detection of CT.

Clinical Study 3. Female Urine Specimen Clinical Study

A retrospective study that used results and remnant female urine samples from a previously completed prospective, multi-center clinical study was conducted to establish the performance characteristics of the Aptima Combo 2 Assay on the Panther System in female urine specimens. Specimens were collected from symptomatic and asymptomatic women (n=2640) enrolled from 17 geographically and ethnically diverse US clinical sites, including family planning clinics, academic center clinics, and public health clinics. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. Of the 2640 subjects enrolled, 42 were withdrawn.

Three specimens were used from each subject (1 first-catch urine and 2 vaginal swabs, in that order). The urine specimens were collected by the subject at the clinic and the vaginal swab specimens were clinician-collected. Urine specimens from each subject were processed into multiple samples for CT/GC testing with different NAATs in accordance with the instructions in the appropriate specimen collection kit package insert. The female urine samples for Aptima Combo 2 Assay testing on the Panther System were split among three external laboratories.

Female urine samples were tested with cleared NAATs to establish a composite comparator algorithm (CCA) result (see Tables 16 and 20). The CCA used urine sample results from up to three reference CT and GC NAATs to generate reference results for each analyte. Subjects were categorized as positive if 2 out of 3 reference NAAT results were positive and negative if 2 out of 3 reference NAAT results were negative. Subjects who could not be categorized as CCA-positive or CCA-negative were excluded from the performance analyses.

Of the 2598 non-withdrawn subjects, 2581 had urine samples tested with the Aptima Combo 2 Assay on the Panther System in accordance with the Aptima Combo 2 Assay package insert instructions. Seventeen subjects had urine samples that were withdrawn or not collected (missing both CT and GC Aptima Combo 2 Assay [Panther System] results). Samples with initial invalid, equivocal, or error results were retested. All 2581 samples had final valid results after required retesting. One sample had a repeat CT equivocal result and one sample had a repeat GC equivocal result.

Of the 2581 subjects that had valid Aptima Combo 2 Assay (Panther System) results, 2580 subjects had a conclusive CT and/or GC composite comparator status and were evaluable for performance; one subject had unknown composite comparator status for both CT and GC and was not evaluable. One evaluable subject had a final equivocal CT result (negative GC result), and one evaluable subject had a final equivocal GC result (negative CT result). Of the 2580 evaluable subjects, 47 were 16 to 17 years of age, 346 were 18 to 20 years of age, 1350 were 21 to 30 years of age, 550 were 31 to 40 years of age, and 287 were >40 years of age.

Of the 2580 evaluable subjects, 2572 subjects were evaluable for performance analyses for CT detection (including one with a final equivocal result). The remaining 8 subjects had an unknown composite comparator status for CT. Of the 2580 evaluable subjects, 2579 subjects were evaluable for performance analyses for GC detection (including one with a final equivocal result). The remaining subject had an unknown composite comparator status for GC. Samples with final equivocal results were categorized as false negative relative to the CCA result (32).

In addition, female urine detected 8.3% fewer CT infections than vaginal and endocervical swab specimens and 12.9% fewer GC infections than vaginal swab specimens and 15.2% fewer GC infections than endocervical swab specimens when compared using the patient infected status (PIS) algorithm.

Chlamydia trachomatis Performance Results

Performance characteristics of the Aptima Combo 2 Assay for CT detection were estimated for each specimen type and are displayed in Tables 5 and 6 combining data from the three clinical studies. Performance was calculated by comparing Panther System results to different infected status algorithms in Clinical Studies 1 and 2 and to a composite comparator algorithm (CCA) in Clinical Study 3 (see Tables 13 through 16 for the CT infected status algorithms). Table 5 shows the sensitivity, specificity, PPV, and NPV of the Aptima Combo 2 Assay for CT detection and the prevalence of CT (based on the infected status) in male urine samples and urethral swab specimens, and in female vaginal swab, endocervical swab, and PCyt specimens.

Table 6 shows the positive percent agreement (PPA) and negative percent agreement (NPA) of the Aptima Combo 2 Assay for CT detection based on the CCA in female urine samples.

Table 5: Performance Characteristics of the Aptima Combo 2 Assay for CT Detection in Female and Male Specimens

Specimen Type[1]	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[2]	Specificity % (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
CVS/PVS	1274	104	18	1149	3	8.4	97.2 (92.1-99.0)	98.5 (97.6-99.0)	85.2 (78.8-90.5)	99.7 (99.3-99.9)
PCyt	1311	112	0	1197	2	8.7	98.2 (93.8-99.5)	100 (99.7-100)	100 (96.9-100)	99.8 (99.4-100)
FS	1254	104	8	1139	3	8.5	97.2 (92.1-99.0)	99.3 (98.6-99.6)	92.9 (87.1-96.7)	99.7 (99.3-99.9)
MS	549	100	4	445	0	18.2	100 (96.3-100)	99.1 (97.7-99.7)	96.2 (90.8-98.9)	100 (99.2-100)
MU	1799	197	3	1589	10	11.5	95.2 (91.3-97.4)	99.8 (99.4-99.9)	98.5 (95.8-99.7)	99.4 (98.9-99.7)

CI = confidence interval, CVS = clinician-collected vaginal swab, FN = false negative, FP = false positive, FS = female endocervical swab, MS = male urethral swab, MU = male urine, NPV = negative predictive value, PCyt = PreservCyt Solution liquid Pap, PPV = positive predictive value, Prev = prevalence, PVS = patient-collected vaginal swab, TN = true negative, TP = true positive.

[1] Male urethral swab, vaginal swab, PreservCyt Solution liquid Pap, and endocervical swab sample results are from Clinical Study 1. Symptomatic male urine sample results are from Clinical Study 2, and asymptomatic male urine sample results are from Clinical Studies 1 and 2.

[2] Score CI.

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

Table 6: Performance Characteristics of the Aptima Combo 2 Assay for CT Detection in Female Urine Samples

Specimen Type[1]	n	CCA+ AC2+	CCA- AC2+	CCA- AC2-	CCA+ AC2-[2]	PPA % (95% CI)[3]	NPA % (95% CI)[3]
FU	2572	174	5	2391	2	98.9 (96.0-99.7)	99.8 (99.5-99.9)

AC2 = Aptima Combo 2 Assay, CCA = composite comparator algorithm, CI = confidence interval, FU = female urine, NPA = negative percent agreement, PPA = positive percent agreement.

[1] Symptomatic and asymptomatic female urine sample results are from Clinical Study 3.

[2] Includes equivocal results from Panther AC2 testing. Equivocal results from AC2 testing are considered indeterminate; a new specimen should be collected.

[3] Score CI.

Table 7 shows the sensitivity, specificity, PPV, and NPV of the Aptima Combo 2 Assay for CT detection and the prevalence of CT (based on the infected status) in male urine samples and urethral swab specimens, and in female vaginal swab, endocervical swab, and PCyt specimens by symptom status. CT prevalence was higher in symptomatic men and women, compared to asymptomatic subjects.

Table 8 shows the PPA and NPA of the Aptima Combo 2 Assay for CT detection based on the CCA in female urine samples by symptom status.

Table 7: Performance Characteristics of the Aptima Combo 2 Assay for CT Detection by Symptom Status in Female and Male Specimens

Specimen Type[1]	Symptom Status	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[2]	Specificity % (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
CVS/PVS	Sym	810	73	8	729	0	9.0	100 (95.0-100)	98.9 (97.9-99.4)	90.1 (82.3-95.5)	100 (99.5-100)
	Asym	464	31	10	420	3	7.3	91.2 (77.0-97.0)	97.7 (95.8-98.7)	75.6 (63.1-86.2)	99.3 (98.1-99.8)
PCyt	Sym	838	76	0	762	0	9.1	100 (95.2-100)	100 (99.5-100)	100 (95.4-100)	100 (99.5-100)
	Asym	473	36	0	435	2	8.0	94.7 (82.7-98.5)	100 (99.1-100)	100 (91.1-100)	99.5 (98.5-99.9)
FS	Sym	794	71	5	718	0	8.9	100 (94.9-100)	99.3 (98.4-99.7)	93.4 (85.9-97.8)	100 (99.5-100)
	Asym	460	33	3	421	3	7.8	91.7 (78.2-97.1)	99.3 (97.9-99.8)	91.7 (79.9-98.0)	99.3 (98.1-99.8)
MS	Sym	238	59	1	178	0	24.8	100 (93.9-100)	99.4 (96.9-99.9)	98.3 (91.5-100)	100 (98.0-100)
	Asym	311	41	3	267	0	13.2	100 (91.4-100)	98.9 (96.8-99.6)	93.2 (82.5-98.5)	100 (98.7-100)
MU	Sym	497	85	1	406	5	18.1	94.4 (87.6-97.6)	99.8 (98.6-100)	98.8 (94.1-100)	98.8 (97.3-99.6)
	Asym	1302	112	2	1183	5	9.0	95.7 (90.4-98.2)	99.8 (99.4-100)	98.2 (94.1-99.8)	99.6 (99.1-99.9)

Asym = asymptomatic, CI = confidence interval, CVS = clinician-collected vaginal swab, FN = false negative, FP = false positive, FS = female endocervical swab, MS = male urethral swab, MU = male urine, NPV = negative predictive value, PCyt = PreservCyt Solution liquid Pap, PPV = positive predictive value, Prev = prevalence, PVS = patient-collected vaginal swab, Sym = symptomatic, TN = true negative, TP = true positive.

[1] Male urethral swab, vaginal swab, PreservCyt Solution liquid Pap, and endocervical swab sample results are from Clinical Study 1. Symptomatic male urine sample results are from Clinical Study 2, and asymptomatic male urine sample results are from Clinical Studies 1 and 2.

[2]Score CI.

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

Table 8: Performance Characteristics of the Aptima Combo 2 Assay for CT Detection by Symptom Status in Female Urine Samples

Specimen Type[1]	Symptom Status	n	CCA+ AC2+	CCA- AC2+	CCA- AC2-	CCA+ AC2-[2]	PPA % (95% CI)[3]	NPA % (95% CI)[3]
FU	Sym	1379	109	2[4]	1267[5]	1	99.1 (95.0-99.8)	99.8 (99.4-100)
	Asym	1193	65	3[6]	1124[7]	1[2]	98.5 (91.9-99.7)	99.7 (99.2-99.9)

AC2 = Aptima Combo 2 Assay, Asym = asymptomatic, CCA = composite comparator algorithm, CI = confidence interval, FU = female urine, NPA = negative percent agreement, PPA = positive percent agreement, Sym = symptomatic.

[1] Symptomatic and asymptomatic female urine sample results are from Clinical Study 3.

[2] Includes equivocal results from Panther AC2 testing. Equivocal results from AC2 testing are considered indeterminate; a new specimen should be collected.

[3] Score CI.

[4] 2/2 subjects had positive CT vaginal swab sample results in both reference NAATs.

[5] 38/1267 subjects had at least one positive CT vaginal swab sample result by a reference NAAT; one or more vaginal swab sample reference results were not available 11/1267 subjects; 1218/1267 subjects had negative vaginal swab sample reference results.

[6] 1/ 3 subject had positive CT vaginal swab sample results in both reference NAATs; 2/3 subjects had negative vaginal swab sample reference results.

[7] 20/1124 subjects had at least one positive CT vaginal swab sample result by a reference NAAT; one or more vaginal swab sample reference results were not available for 11/1124 subjects; 1093/1124 subjects had negative vaginal swab sample reference results.

Neisseria gonorrhoeae Performance Results

Performance characteristics of the Aptima Combo 2 Assay for GC detection were estimated for each specimen type and are displayed in Tables 9 and 10 combining data from the three clinical studies. The infected status algorithm differed among the three clinical studies (see Tables 17 through 20 for the GC infected status algorithms). Table 9 shows the sensitivity, specificity, PPV, and NPV of the Aptima Combo 2 Assay for GC detection and the prevalence of GC (based on

the infected status) in male urine samples and urethral swab specimens, and in female vaginal swab, endocervical swab, and PCyt specimens.

Table 10 shows the PPA and NPA of the Aptima Combo 2 Assay for GC detection based on the CCA in female urine samples.

Table 9: Performance Characteristics of the Aptima Combo 2 Assay for GC Detection in Female and Male Specimens

Specimen Type[1]	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[2]	Specificity % (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
CVS/PVS	1258	42	5	1210	1	3.4	97.7 (87.9-99.6)	99.6 (99.0-99.8)	89.4 (78.6-96.1)	99.9 (99.6-100)
PCyt	1293	43	0	1250	0	3.3	100 (91.8-100)	100 (99.7-100)	100 (92.1-100)	100 (99.7-100)
FS	1238	42	2	1194	0	3.4	100 (91.6-100)	99.8 (99.4-100)	95.5 (85.4-99.4)	100 (99.7-100)
MS	546	34	0	512	0	6.2	100 (89.8-100)	100 (99.3-100)	100 (90.2-100)	100 (99.3-100)
MU	1797	75	5	1716	1	4.2	98.7 (92.9-99.8)	99.7 (99.3-99.9)	93.8 (86.7-97.8)	99.9 (99.7-100)

CI = confidence interval, CVS = clinician-collected vaginal swab, FN = false negative, FP = false positive, FS = female endocervical swab, MS = male urethral swab, MU = male urine, NPV = negative predictive value, PCyt = PreservCyt Solution liquid Pap, PPV = positive predictive value, Prev = prevalence, PVS = patient-collected vaginal swab, TN = true negative, TP = true positive.

[1] Vaginal swab, PreservCyt Solution liquid Pap, endocervical swab, and male urethral swab sample results are from Clinical Study 1. Symptomatic male urine sample results are from Clinical Study 2, and asymptomatic male urine sample results are from Clinical Studies 1 and 2.

[2] Score CI.

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

Table 10: Performance Characteristics of the Aptima Combo 2 Assay for GC Detection in Female Urine Samples

Specimen Type[1]	n	CCA+ AC2+	CCA- AC2+	CCA- AC2-	CCA+ AC2-[2]	PPA % (95% CI)[3]	NPA % (95% CI)[3]
FU	2579	28	0	2550	1	96.6 (82.8-99.4)	100 (99.8-100)

AC2 = Aptima Combo 2 Assay, CCA = composite comparator algorithm, CI = confidence interval, FU = female urine, NPA = negative percent agreement, PPA = positive percent agreement.

[1] Symptomatic and asymptomatic female urine sample results are from Clinical Study 3.

[2] Includes equivocal results from Panther AC2 testing. Equivocal results from AC2 testing are considered indeterminate; a new specimen should be collected.

[3] Score CI.

Table 11 shows the sensitivity, specificity, PPV, and NPV of the Aptima Combo 2 Assay for GC detection and the prevalence of GC (based on the infected status) in male urine samples and urethral swab specimens, and in female vaginal swab, endocervical swab, and PCyt specimens by symptom status. GC prevalence was higher in symptomatic men but similar in symptomatic and asymptomatic women.

Table 12 shows the PPA and NPA of the Aptima Combo 2 Assay for CT detection based on the CCA in female urine samples by symptom status.

Table 11: Performance Characteristics of the Aptima Combo 2 Assay for GC Detection by Symptom Status in Female and Male Specimens

Specimen Type[1]	Symptom Status	n	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[2]	Specificity % (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
CVS/PVS	Sym	802	27	4	771	0	3.4	100 (87.5-100)	99.5 (98.7-99.8)	87.1 (72.6-96.1)	100 (99.6-100)
	Asym	456	15	1	439	1	3.5	93.8 (71.7-98.9)	99.8 (98.7-100)	93.8 (74.0-99.8)	99.8 (98.9-100)
PCyt	Sym	829	27	0	802	0	3.3	100 (87.5-100)	100 (99.5-100)	100 (88.0-100)	100 (99.6-100)
	Asym	464	16	0	448	0	3.4	100 (80.6-100)	100 (99.1-100)	100 (81.3-100)	100 (99.3-100)
FS	Sym	785	26	1	758	0	3.3	100 (87.1-100)	99.9 (99.3-100)	96.3 (82.4-99.9)	100 (99.5-100)
	Asym	453	16	1	436	0	3.5	100 (80.6-100)	99.8 (98.7-100)	94.1 (74.3-99.8)	100 (99.3-100)
MS	Sym	236	31	0	205	0	13.1	100 (89.0-100)	100 (98.2-100)	100 (89.5-100)	100 (98.3-100)
	Asym	310	3	0	307	0	1.0	100 (43.9-100)	100 (98.8-100)	100 (44.4-100)	100 (99.3-100)
MU	Sym	497	66	1	430	0	13.3	100 (94.5-100)	99.8 (98.7-100)	98.5 (92.3-100)	100 (99.2-100)
	Asym	1300	9	4	1286	1	0.8	90.0 (59.6-98.2)	99.7 (99.2-99.9)	69.2 (45.6-91.7)	99.9 (99.7-100)

Asym = asymptomatic, CI = confidence interval, CVS = clinician-collected vaginal swab, FN = false negative, FP = false positive, FS = female endocervical swab, MS = male urethral swab, MU = male urine, NPV = negative predictive value, PCyt = PreservCyt Solution liquid Pap, PPV = positive predictive value, Prev = prevalence, PVS = patient-collected vaginal swab, Sym = symptomatic, TN = true negative, TP = true positive.

[1] Vaginal swab, PreservCyt Solution liquid Pap, endocervical swab, and male urethral swab sample results are from Clinical Study 1. Symptomatic male urine sample results are from Clinical Study 2, and asymptomatic male urine sample results are from Clinical Studies 1 and 2.

[2] Score CI.

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

Table 12: Performance Characteristics of the Aptima Combo 2 Assay for GC Detection by Symptom Status in Female Urine Samples

Specimen Type[1]	Symptom Status	n	CCA+ AC2+	CCA- AC2+	CCA- AC2-	CCA+ AC2-[2]	PPA % (95% CI)[3]	NPA % (95% CI)[3]
FU	Sym	1383	19	0	1363[4]	1	95.0 (76.4-99.1)	100 (99.7-100)
	Asym	1196	9	0	1187[5]	0	100 (70.1-100)	100 (99.7-100)

AC2 = Aptima Combo 2 Assay, Asym = asymptomatic, CCA = composite comparator algorithm, CI = confidence interval, FU = female urine, NPA = negative percent agreement, PPA = positive percent agreement, Sym = symptomatic.

[1] Symptomatic and asymptomatic female urine sample results are from Clinical Study 3.

[2] Includes equivocal results from Panther AC2 testing. Equivocal results from AC2 testing are considered indeterminate; a new specimen should be collected.

[3] Score CI.

[4] 5/1363 subjects had at least one positive GC vaginal swab sample result by a reference NAAT; one or more vaginal swab sample reference results were not available for 11/1363 subjects; 1347/1363 subjects had negative vaginal swab sample reference results.

[5] 6/1187 subjects had at least one positive GC vaginal swab sample result by a reference NAAT; one or more vaginal swab sample reference results were not available for 11/1187 subjects; 1170/1187 asymptomatic subjects had negative vaginal swab sample reference results.

Chlamydia trachomatis Infected Status Tables

The frequency of test outcomes from reference NAAT and investigational Panther System testing is summarized in Tables 13 through 16 for CT.

Table 13: Clinical Study 1. CT Infected Status for Performance Evaluation in Female Vaginal Swab, PreservCyt Solution Liquid Pap, and Endocervical Swab Samples

CT Infected Status	Assay Results							Symptom Status	
	AC2 Tigris		ACT Tigris		AC2 Panther				
	PCyt	FU	PCyt	FU	CVS/PVS	PCyt	FS	Sym	Asym
Infected	+	+	+	+	+	+	+	62	26
Infected	+	+	+	+	+	+	-	0	1
Infected	+	+	+	+	+	+	NA	3	0
Infected	+	+	+	+	+	-	+	0	2
Infected	+	+	+	+	-	+	+	0	1
Infected	+	+	+	+	NA	+	+	1	1
Infected	+	+	+	+	NA	+	NA	2	1
Infected	+	-	+	+	+	+	+	4	1
Infected	+	-	+	+	NA	+	NA	0	1
Infected	+	-	+	-	+	+	+	4	0
Infected	+	-	+	-	-	+	-	0	1
Infected	+	-	+	-	NA	+	+	0	1
Infected	+	NA	+	NA	+	+	+	0	1
Infected	+	NA	+	NA	-	+	-	0	1
Infected[1]	-	+	-	+	+	-	+	1	0
Infected[1]	-	+	-	+	+	-	-	2	0
Infected[1]	-	+	-	+	-	-	-	1	1
Not Infected	+	-	-	-	-	-	-	0	2
Not Infected	-	+	-	-	-	-	-	1	0
Not Infected	-	-	+	-	+	-	+	0	1
Not Infected	-	-	+	-	-	-	-	5	0
Not Infected	-	-	-	+	+	-	-	0	1
Not Infected	-	-	-	+	+	-	NA	0	1
Not Infected	-	-	-	+	-	-	-	1	3
Not Infected	-	-	-	-	+	-	+	1	0
Not Infected	-	-	-	-	+	-	-	2	7
Not Infected	-	-	-	-	+	-	NA	2	0
Not Infected	-	-	-	-	-	-	+	2	2
Not Infected	-	-	-	-	-	-	-	680	396
Not Infected	-	-	-	-	-	-	NA	29	8
Not Infected	-	-	-	-	-	NA	-	1	0
Not Infected	-	-	-	-	NA	-	-	17	4
Not Infected	-	-	-	-	NA	-	NA	8	1
Not Infected	-	NA	-	-	-	-	-	8	6
Not Infected	-	NA	-	-	-	-	NA	0	1
Not Infected	NA	-	-	-	-	-	-	0	1
Not Infected	NA	-	-	-	-	-	NA	1	0
Not Infected	NA	-	-	-	NA	-	+	1	0

AC2 = Aptima Combo 2 Assay, ACT = Aptima CT Assay, Asym = asymptomatic, CVS = clinician-collected vaginal swab, FS = female endocervical swab, FU = female urine, NA = result not available, Panther = Panther System, PCyt = PreservCyt Solution liquid Pap, PVS = patient-collected vaginal swab, Sym = symptomatic, Tigris = Tigris DTS System.

[1] For the evaluation of the non-urine specimen types, the specimens were considered non-infected.

Table 14: Clinical Study 1. CT Infected Status for Performance Evaluation in Male Urethral Swab Samples

CT Infected Status	Assay Results					Symptom Status	
	AC2 DTS		ACT Tigris		AC2 Panther		
	MS	MU	MS	MU	MS	Sym	Asym
Infected	+	+	+	+	+	50	37
Infected	+	+	+	+	NA	4	1
Infected	+	+	+	-	+	2	0
Infected	+	-	+	+	+	4	2
Infected	+	-	+	-	+	3	2
Not Infected	+	+	-	-	-	0	1
Not Infected	+	-	-	-	+	0	1
Not Infected	+	-	-	-	-	1	1
Not Infected	-	-	+	-	-	3	2
Not Infected	-	-	-	+	-	1	1
Not Infected	-	-	-	-	+	1	2
Not Infected	-	-	-	-	-	173	262
Not Infected	-	-	-	-	NA	10	9
Not Infected	NA	-	-	-	NA	1	2

AC2 = Aptima Combo 2 Assay, ACT = Aptima CT Assay, Asym = asymptomatic, DTS = DTS Systems, MS = male urethral swab, MU = male urine, NA = result not available, Panther = Panther System, Sym = symptomatic, Tigris = Tigris DTS System.

Table 15: Clinical Study 1 and Clinical Study 2. CT Infected Status for Performance Evaluation in Male Urine Samples

CT Infected Status	Assay Results							Symptom Status	
	AC2[1]		ACT Tigris		NAAT 1[3]	NAAT 2[3]	AC2 Panther		
	MS	MU	MS	MU	MU	MU	MU	Sym	Asym
Clinical Study 1									
Infected	+	+	+	+			+		38
Infected	+	-	+	+			+		2
Infected	+	-	+	-			-		2
Clinical Study 2									
Infected	+	+			+	+	+	73	66
Infected	+	+			+	+	-	2	1
Infected	+	+			+	-	+	0	1
Infected	+	+			+	NA	+	0	1
Infected	+	+			-	+	+	3	0
Infected	+	+			-	+	-	0	1
Infected	+	-			+	+	+	4	0
Infected	+	-			+	+	-	3	0
Infected	+	=			-	+	-	0	1
Infected	-	+			+	+	+	5	4
Clinical Study 1									
Not Infected	+	+	-	-			-		1
Not Infected	+	-	-	-			-		2
Not Infected	-	-	+	-			-		2

Table 15: Clinical Study 1 and Clinical Study 2. CT Infected Status for Performance Evaluation in Male Urine Samples (Continued)

CT Infected Status	Assay Results							Symptom Status	
	AC2[1]		ACT Tigris		NAAT 1[3]	NAAT 2[3]	AC2 Panther		
	MS	MU	MS	MU	MU	MU	MU	Sym	Asym
Not Infected	-	-	-	+			+		1
Not Infected	-	-	-	-			-		273
Not Infected	NA	-	-	-			-		2
Clinical Study 2									
Not Infected	+	-			-	-	-	1	6
Not Infected	-	+			-	-	+	0	1
Not Infected	-	-			+	-	+	1	0
Not Infected	-	-			+	-	-	0	2
Not Infected	-	-			-	-	-	388	874
Not Infected	-	-			-	=	-	0	1
Not Infected	-	-			-	NA	-	10	18
Not Infected	-	-			NA	-	-	1	2
Not Infected	-	NA			-	-	-	2	0
Not Infected	NA	-			-	-	-	4	0

AC2 = Aptima Combo 2 Assay, ACT = Aptima CT Assay, Asym = asymptomatic, MS = male urethral swab, MU = male urine, NA = result not available, Panther = Panther System, Sym = symptomatic, Tigris = Tigris DTS System.

The equal symbol (=) represents an equivocal result.

[1] Male urethral swab and male urine samples were tested with the Aptima Combo 2 Assay on the DTS Systems in Clinical Study 1 and on the Tigris DTS System in Clinical Study 2.

[2] Male urethral swab and male urine samples were tested with the Aptima CT Assay on the Tigris DTS System in Clinical Study 1.

[3] Male urine samples were tested with two FDA-cleared CT NAATs in Clinical Study 2.

Note. Data from asymptomatic men in Clinical Study 1 are combined with data from Clinical Study 2.

Table 16: Clinical Study 3. CT Composite Comparator Status for Performance Evaluation in Female Urine Samples

Composite Comparator Status	Assay Results				Symptom Status	
	NAAT 1	NAAT 2	NAAT 3	AC2 Panther		
	FU	FU	FU	FU	Sym	Asym
Positive	+	+	NR	+	101	61
Positive	+	+	NR	-	1	0
Positive	+	+	NR	=	0	1
Positive	+	-	+	+	4	4
Positive	-	+	+	+	3	0
Positive	=	+	+	+	1	0
Negative	-	+	-	+	1	0
Negative	-	+	-	-	3	1
Negative	-	-	NR	+	1	3
Negative	-	-	NR	-	1261	1119
Negative	-	NA	-	-	1	1
Negative	NA	-	-	-	2	3

Asym = asymptomatic, FU = female urine, NA = result not available, NR = not required, AC2 Panther = Aptima Combo 2 assay on the Panther system, Sym = symptomatic.

The equal symbol (=) represents final equivocal result.

Neisseria gonorrhoeae Infected Status Tables

The frequency of test outcomes from reference NAAT and investigational Panther System testing is summarized in Tables 17 through 20 for GC.

Table 17: Clinical Study 1. GC Infected Status for Performance Evaluation in Female Vaginal Swab, PreservCyt Solution Liquid Pap, and Endocervical Swab Samples

GC Infected Status	Assay Results							Symptom Status	
	AC2 Tigris		AGC Tigris		AC2 Panther				
	PCyt	FU	PCyt	FU	CVS/PVS	PCyt	FS	Sym	Asym
Infected	+	+	+	+	+	+	+	22	10
Infected	+	+	+	+	+	+	NA	1	0
Infected	+	+	+	-	+	+	+	1	0
Infected	+	+	+	=	+	+	+	0	1
Infected	+	-	+	-	+	+	+	3	3
Infected	+	-	+	-	-	+	+	0	1
Infected	+	NA	+	NA	+	+	+	0	1
Not Infected	+	NA	-	-	-	=	-	0	1
Not Infected	-	-	NA	NA	+	-	+	0	1
Not Infected	-	-	NA	NA	+	-	-	3	0
Not Infected	-	-	NA	NA	+	-	NA	1	0
Not Infected	-	-	NA	NA	-	-	+	1	0
Not Infected	-	-	NA	NA	-	-	-	736	429
Not Infected	-	-	NA	NA	-	-	=	1	0
Not Infected	-	-	NA	NA	-	-	NA	32	9
Not Infected	-	-	NA	NA	-	NA	-	1	0
Not Infected	-	-	NA	NA	NA	-	-	18	6
Not Infected	-	-	NA	NA	NA	-	NA	10	3

AC2 = Aptima Combo 2 Assay, AGC = Aptima GC Assay, Asym = asymptomatic, CVS = clinician-collected vaginal swab, FS = female endocervical swab, FU = female urine, NA = result not available, Panther = Panther System, PCyt = PreservCyt Solution liquid Pap, PVS = patient-collected vaginal swab, Sym = symptomatic, Tigris = Tigris DTS System.

The equal symbol (=) represents an equivocal result on repeat testing.

Table 18: Clinical Study 1. GC Infected Status for Performance Evaluation in Male Urethral Swab Samples

GC Infected Status	Assay Results					Symptom Status	
	AC2 DTS		AGC DTS		AC2 Panther		
	MS	MU	MS	MU	MS	Sym	Asym
Infected	+	+	+	+	+	30	2
Infected	+	+	+	+	NA	0	1
Infected	+	-	+	-	+	1	1
Infected	NA	+	NA	+	NA	1	0
Not Infected	-	-	NA	NA	-	205	307
Not Infected	-	-	NA	NA	NA	14	9

AC2 = Aptima Combo 2 Assay, AGC = Aptima GC Assay, Asym = asymptomatic, DTS = DTS Systems, MS = male urethral swab, MU = male urine, NA = result not available, Panther = Panther System, Sym = symptomatic.

Table 19: Clinical Study 1 and Clinical Study 2. GC Infected Status for Performance Evaluation in Male Urine Samples

GC Infected Status	Assay Results							Symptom Status	
	AC2[1]		AGC DTS[2]		NAAT 1[3]	NAAT 2[3]	AC2 Panther		
	MS	MU	MS	MU	MU	MU	MU	Sym	Asym
Clinical Study 1									
Infected	+	+	+	+			+		3
Infected	+	-	+	-			-		1
Clinical Study 2									
Infected	+	+			+	+	+	63	4
Infected	+	+			+	NA	+	1	1
Infected	-	+			+	-	+	0	1
Infected	NA	+			+	+	+	2	0
Clinical Study 1									
Not Infected	-	-	NA	NA			+		2
Not Infected	-	-	NA	NA			-		314
Clinical Study 2									
Not Infected	+	-			-	-	-	2	4
Not Infected	-	+			-	-	+	0	1
Not Infected	-	-			+	-	-	6	2
Not Infected	-	-			-	+	-	1	0
Not Infected	-	-			-	-	+	1	1
Not Infected	-	-			-	-	-	407	945
Not Infected	-	-			-	NA	-	9	19
Not Infected	-	-			NA	-	-	1	2
Not Infected	-	NA			-	-	-	2	0
Not Infected	NA	-			-	-	-	2	0

AC2 = Aptima Combo 2 Assay, AGC = Aptima GC Assay, Asym = asymptomatic, DTS = DTS Systems, MS = male urethral swab, MU = male urine, NA = result not available, Panther = Panther System, Sym = symptomatic.

[1] Male urethral swab and male urine samples were tested with the Aptima Combo 2 Assay on the DTS Systems in Clinical Study 1 and on the Tigris DTS System in Clinical Study 2.

[2] Male urethral swab and male urine samples were tested with the Aptima GC Assay on the DTS Systems in Clinical Study 1.

[3] Male urine samples were tested with two FDA-cleared GC NAATs in Clinical Study 2.

Note. Data from asymptomatic men in Clinical Study 1 are combined with data from Clinical Study 2.

Table 20: Clinical Study 3. GC Composite Comparator Status for Performance Evaluation in Female Urine Samples

Composite Comparator Status	Assay Results				Symptom Status	
	NAAT 1 FU	NAAT 2 FU	NAAT 3 FU	AC2 Panther FU	Sym	Asym
Positive	+	+	NR	+	19	9
Positive	=	+	+	=	1	0
Negative	-	-	NR	-	1360	1183
Negative	-	NA	-	-	1	1
Negative	NA	-	-	-	2	3

Asym = asymptomatic, FU = female urine, NA = result not available, NR = not required, AC2 Panther = Aptima Combo 2 assay on the Panther system, Sym = symptomatic.
The equal symbol (=) represents final equivocal result.

RLU Distribution of Aptima Combo 2 Controls

The distribution of the RLU values for the Aptima Combo 2 controls is presented in Table 21 from all valid Panther System runs performed during Clinical Study 1, Clinical Study 2, and Clinical Study 3.

Table 21: RLU Distribution of Aptima Combo 2 Controls

Control	Statistic	Total RLU (x1000)		
		Clinical Study 1	Clinical Study 2	Clinical Study 3
Positive Control, CT/ Negative Control, GC	N	66	23	41
	Maximum	1335	1258	1577
	Median	1081.5	1135.0	1091.0
	Minimum	624	910	771
	CV%	11.2	7.5	13.5
Positive Control, GC/ Negative Control, CT	N	66	23	41
	Maximum	1241	1311	1308
	Median	1172.0	1174.0	1060.0
	Minimum	1063	1082	905
	CV%	3.2	4.9	8.9

Reproducibility Studies

Reproducibility of the Aptima Combo 2 Assay on the Panther System was evaluated in two different studies using panel members created with Specimen Transport Medium (STM) in Reproducibility Study 1 and using panel members created with clinical urine specimens in Reproducibility Study 2.

Reproducibility Study 1

Aptima Combo 2 Assay reproducibility was evaluated with panel members created using STM at three external US laboratories using the Panther System. Testing was performed using one lot of assay reagents and a total of six operators (two at each site). Testing was performed over at least 10 days at each site. The negative panel member consisted of STM and positive panel members were created by spiking STM with lysate from CT and/or GC organisms to result in panel members with expected targeted concentrations. Table 22 shows the CT and GC concentrations for each panel member and the mean, standard deviation (SD), and coefficient of variation (CV) of the RLU data for each panel member between-sites, between-operators,

between-days, between-runs, within-runs, and overall. Percent agreement with expected results is also shown. Only samples with valid results were included in the analyses.

Table 22: Reproducibility Study 1 Data

Target Concentration		Agreed/N	Agmt (%)	Mean RLU (x1000)	Between-Sites		Between-Operators		Between-Days		Between-Runs		Within-Runs		Total	
CT (IFU/mL)	GC (CFU/mL)				SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
0	0	180/180	100	6	1.0	17.5	0.5	8.1	0.2	3.7	0.5	8.2	1.5	24.4	1.9	32.4
0.25	0	180/180	100	1207	45.0	3.7	17.3	1.4	0.0	0.0	35.1	2.9	66.9	5.5	89.7	7.4
2.5	0	180/180	100	1272	41.3	3.2	19.2	1.5	0.0	0.0	31.0	2.4	36.8	2.9	66.3	5.2
25	0	180/180	100	1292	43.7	3.4	14.9	1.2	7.7	0.6	35.1	2.7	36.3	2.8	68.8	5.3
1000	0	180/180	100	1294	48.1	3.7	14.3	1.1	26.8	2.1	29.6	2.3	34.8	2.7	73.0	5.6
0	0.25	180/180	100	589	92.2	15.7	19.9	3.4	28.1	4.8	21.2	3.6	44.8	7.6	110.2	18.7
0	12.5	179/179	100	1251	163.5	13.1	0.0	0.0	15.1	1.2	31.5	2.5	29.8	2.4	169.8	13.6
0	125	180/180	100	1295	168.3	13.0	6.7	0.5	33.4	2.6	21.1	1.6	33.3	2.6	176.2	13.6
0	1250	180/180	100	1309	166.5	12.7	0.0	0.0	28.4	2.2	27.6	2.1	31.2	2.4	173.9	13.3
0	2500	179/179	100	1305	170.9	13.1	11.4	0.9	30.4	2.3	15.2	1.2	32.2	2.5	177.5	13.6
2.5	125	178/178	100	2513	123.9	4.9	24.6	1.0	24.0	1.0	57.5	2.3	52.4	2.1	150.3	6.0
2.5	2500	180/180	100	2515	123.5	4.9	6.5	0.3	33.8	1.3	39.3	1.6	59.4	2.4	146.6	5.8
1000	125	179/179	100	2524	117.4	4.6	35.2	1.4	52.1	2.1	28.9	1.1	54.7	2.2	146.8	5.8
1000	2500	180/180	100	2525	118.2	4.7	21.6	0.9	38.7	1.5	54.8	2.2	48.5	1.9	145.9	5.8

Agmt = agreement, CFU = colony-forming unit, CV = coefficient of variation, IFU = inclusion-forming unit, RLU = relative light unit, SD = standard deviation.

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.

Reproducibility Study 2

Aptima Combo 2 Assay reproducibility was evaluated with panel members created using clinical urine specimens at two external US laboratories and in-house using the Panther System. Testing was performed using one lot of assay reagents and a total of six operators (two at each site). Testing was performed over at least 10 days at each site. The negative panel member consisted of negative urine and the positive panel members were created by spiking negative urine with lysate from CT and/or GC organisms to result in panel members with expected targeted concentrations. Table 23 shows the CT and GC concentrations for each panel member and the mean, SD, and CV of the RLU data for each panel member between-sites, between-operators, between-days, between-runs, within-runs, and overall. Percent agreement with expected results is also shown. Only samples with valid results were included in the analyses.

Table 23: Reproducibility Study 2 Data

Target Concentration		Agreed/N	Agmt (%)	Mean RLU (x1000)	Between-Sites		Between-Operators		Between-Days		Between-Runs		Within-Runs		Total	
CT (IFU/mL)	GC (CFU/mL)				SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
0	0	178/180	98.9	6	1.2	19.0	0.0	0.0	0.0	0.0	0.0	0.0	8.2	131.7	8.3	133.0
0.25	0	180/180	100	1202	92.4	7.7	0.0	0.0	0.0	0.0	62.9	5.2	50.3	4.2	122.6	10.2
2.5	0	178/178	100	1185	90.9	7.7	0.0	0.0	0.0	0.0	53.8	4.5	34.6	2.9	111.1	9.4
25	0	180/180	100	1265	97.4	7.7	18.9	1.5	0.0	0.0	62.4	4.9	35.1	2.8	122.4	9.7
1000	0	180/180	100	1278	101.9	8.0	15.7	1.2	20.6	1.6	61.4	4.8	31.8	2.5	125.9	9.8
0	0.25	177/179	98.9	422	40.3	9.5	21.9	5.2	27.6	6.5	35.3	8.4	72.7	17.2	96.9	23.0
0	12.5	179/180	99.4	1142	11.9	1.0	0.0	0.0	44.4	3.9	37.3	3.3	75.8	6.6	96.2	8.4
0	125	180/180	100	1224	31.4	2.6	13.0	1.1	11.1	0.9	19.8	1.6	34.3	2.8	53.4	4.4
0	1250	180/180	100	1263	16.7	1.3	9.4	0.7	21.0	1.7	14.0	1.1	30.6	2.4	44.1	3.5
0	2500	180/180	100	1309	20.7	1.6	13.4	1.0	0.0	0.0	21.7	1.7	25.3	1.9	41.4	3.2
2.5	125	180/180	100	2468	71.9	2.9	31.5	1.3	21.7	0.9	64.8	2.6	44.4	1.8	113.1	4.6
2.5	2500	180/180	100	2453	76.2	3.1	30.9	1.3	0.0	0.0	62.5	2.5	51.6	2.1	115.4	4.7
1000	125	179/179	100	2504	74.0	3.0	38.5	1.5	0.0	0.0	59.1	2.4	39.1	1.6	109.4	4.4
1000	2500	180/180	100	2357	79.1	3.4	0.0	0.0	0.0	0.0	74.2	3.1	55.2	2.3	121.7	5.2

Agmt = agreement, CFU = colony-forming unit, CV = coefficient of variation, IFU = inclusion-forming unit, RLU = relative light unit, SD = standard deviation.

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.

Panther System Analytical Performance

Analytical Sensitivity Study

Chlamydia trachomatis analytical sensitivity (limit of detection) was determined by testing dilutions of CT organisms in the Aptima Combo 2 Assay. The analytical sensitivity claim for the assay is 1 IFU/assay (7.25 IFU/swab, 9.75 IFU/mL PreservCyt Solution liquid Pap, 5.0 IFU/mL urine). However, dilutions of less than 1 IFU/assay tested positive in the Aptima Combo 2 Assay for the following 12 serovars: D, E, F, G, H, I, J, K, L1, L2, L2a, and L3 (≥95% positivity was observed in samples containing CT concentrations of 1.89 IFU/mL).

Neisseria gonorrhoeae analytical sensitivity (limit of detection) was determined by testing dilutions of GC organisms in the Aptima Combo 2 Assay. The analytical sensitivity claim for the assay is 50 cells/assay (362 cells/swab, 488 cells/mL PreservCyt Solution liquid Pap, 250 cells/mL urine). However, dilutions of less than 50 cells/assay tested positive in the Aptima Combo 2 Assay for 30 different strains of GC (≥95% positivity was observed in samples containing GC concentrations of 0.36 cells/mL).

Analytical Specificity

The Aptima Combo 2 Assay formulation for the Panther System has not changed from that used with DTS® Systems or the Tigris® DTS System. The analytical specificity study was conducted on DTS Systems. A total of 154 culture isolates were evaluated using the Aptima Combo 2 Assay. These isolates included 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, parasites, and viruses. All organisms except *C. psittaci*, *C. pneumoniae*, and the viruses were tested at 1.0 x 10^6 cells/assay in STM. 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. Only CT and GC samples produced positive results in the Aptima Combo 2 Assay. The list of organisms tested is shown in Table 24.

Table 24: 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*

"(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 24: Analytical Specificity (Continued)

Organism	Organism	Organism
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.

Interfering Substances

The Aptima Combo 2 Assay formulation for the Panther System has not changed from that used with DTS Systems or the Tigris DTS System. Blood interference was evaluated on the Panther System and the results of this testing indicated that blood does not interfere with Aptima Combo 2 Assay performance.

Aptima Combo 2 Assay performance in the presence of potentially interfering substances was tested on DTS Systems. 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). 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.

Within Laboratory Precision Study

Aptima Combo 2 Assay precision was evaluated at Hologic using the Panther System. Testing was performed using three Panther Systems and three lots of assay reagents. Testing was performed over 24 days.

Reproducibility panel members were created using negative PreservCyt Solution liquid Pap specimens and STM. The positive panel members were created by spiking CT and/or GC organisms to the targeted concentrations shown in Table 25.

For each panel member, Table 25 presents mean RLU, between-instrument, between-lot, between-run, within-run, and overall variation as SD and percent CV. Percent agreement with expected results is also shown.

Table 25: Within Laboratory Precision Data

Matrix	Target Concentration		Agreed/N	Agrmt (%)	Mean RLU (x1000)	Between-Instruments		Between-Lots		Between-Runs		Within-Runs		Total	
	CT (IFU/mL)	GC (CFU/mL)				SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
STM	0	0	96/96	100	6	0.1	1.0	0.9	13.5	0.0	0.0	1.0	15.7	1.3	20.1
	0.25	0	95/95	100	1226	70.0	5.7	20.0	1.6	8.4	0.7	47.1	3.8	87.1	7.1
	2.5	0	96/96	100	1249	78.0	6.2	6.1	0.5	0.0	0.0	32.9	2.6	84.8	6.8
	25	0	95/95	100	1268	72.9	5.7	15.3	1.2	0.0	0.0	39.6	3.1	84.3	6.6
	0	12.5	96/96	100	1081	18.4	1.7	28.6	2.6	0.0	0.0	26.7	2.5	43.2	4.0
	0	125	96/96	100	1266	29.8	2.4	0.0	0.0	8.9	0.7	27.6	2.2	41.6	3.3
	0	1250	96/96	100	1309	29.4	2.2	0.0	0.0	9.8	0.8	31.8	2.4	44.4	3.4
	2.5	125	96/96	100	2456	86.6	3.5	0.0	0.0	0.0	0.0	53.0	2.2	101.5	4.1
	2.5	2500	96/96	100	2509	73.1	2.9	0.0	0.0	19.8	0.8	46.8	1.9	89.0	3.5
	1000	2500	96/96	100	2496	31.7	1.3	6.1	0.2	0.0	0.0	193.7	7.8	196.3	7.9
	1000	125	96/96	100	2471	83.6	3.4	9.4	0.4	0.0	0.0	52.4	2.1	99.1	4.0
PCyt	0	0	96/96	100	7	0.0	0.0	0.8	11.7	0.0	0.0	1.5	22.4	1.7	24.7
	0.25	0	96/96	100	1113	92.3	8.3	30.1	2.7	0.0	0.0	63.6	5.7	116.0	10.4
	2.5	0	96/96	100	1194	62.5	5.2	24.8	2.1	0.0	0.0	47.0	3.9	82.1	6.9
	25	0	95/95	100	1222	65.1	5.3	26.4	2.2	14.7	1.2	35.0	2.9	79.8	6.5
	0	12.5	93/93	100	994	33.3	3.3	36.9	3.7	16.0	1.6	26.2	2.6	58.4	5.9
	0	125	95/95	100	1189	40.1	3.4	4.5	0.4	10.9	0.9	21.4	1.8	47.0	4.0
	0	1250	95/95	100	1239	37.7	3.0	7.5	0.6	13.6	1.1	18.0	1.5	44.6	3.6
	2.5	125	95/95	100	2333	99.7	4.3	35.3	1.5	12.6	0.5	48.9	2.1	117.2	5.0

Agrmt = agreement, CFU = colony-forming unit, CV = coefficient of variation, IFU = inclusion-forming unit, N = number of samples, PCyt = PreservCyt Solution liquid Pap, RLU = relative light unit, SD = standard deviation, STM = specimen transport medium.
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.

Carryover Studies for the Panther System

Two studies were conducted to evaluate carryover on the Panther System. In the first study, carryover was assessed in multiple runs on three Panther Systems with 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 within the run. High titer samples were made using GC rRNA spiked into STM to give a final concentration equivalent to 2.5×10^5 CFU/mL. Five runs were performed on each of three Panther Systems. Carryover was calculated from a total of 2938 valid negative results. The overall carryover rate from this study was 0% with a 95% confidence interval of 0–0.1%.

The second carryover study was conducted on one Panther System with high titer GC positive samples (GC rRNA spiked into STM at the equivalent of 2.5×10^5 CFU/mL) alternately processed with negative samples in a checkerboard format. Five checkerboard runs were performed. The overall carryover rate from this study was 0.74% (1/135 negative samples).

Specimen Stability Studies

Specimen stability was evaluated using the DTS Systems and/or the Tigris DTS System.

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 IFU 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 both 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, 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.

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