GEN-PROBE® APTIMA® Assay for *Chlamydia trachomatis*

For *in vitro* diagnostic use.

Intended Use

The APTIMA Assay for *Chlamydia trachomatis* is a target amplification nucleic acid probe test that utilizes target capture for the *in vitro* qualitative detection of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) in clinician-collected endocervical, vaginal and male urethral swab specimens, patient-collected vaginal swab specimens[1], and female and male urine specimens. The assay may be used to test specimens from symptomatic and asymptomatic individuals to aid in the diagnosis of chlamydial urogenital disease.

Summary and Explanation of the Test

Chlamydia trachomatis infections are one of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 929,462 new cases of CT infections were reported in 2004 (4).

Chlamydiae are nonmotile, gram-negative, obligate intracellular bacteria. The CT species is comprised of fifteen serovars that can cause disease in humans. The serovars D through K are the major cause of genital chlamydial infections in men and women (19). *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and pelvic inflammatory disease (3, 13, 21, 22). *C. trachomatis* infections are often asymptomatic in both males and females. Children born to infected mothers are at significantly higher risk for inclusion conjunctivitis and chlamydial pneumonia (1, 10, 20).

Historically, several methods for CT detection have been utilized in the clinical laboratory, including cell culture, direct fluorescent antibody testing, and enzyme immunoassay. More recent methodologies for CT detection include direct DNA probe assays and nucleic acid amplification tests (NAATs). Cell culture was once considered to be the “gold standard” for detection of CT. Culture is quite specific, but recent publications have demonstrated that NAATs have a higher clinical sensitivity than culture (2, 8, 14, 23). Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe and NAATs.

First generation NAATs for CT have technological issues that have limited their performance. These issues include cumbersome specimen processing and specimen inhibition that can yield false negative results (6, 12, 15, 18, 24, 26). The GEN-PROBE APTIMA Assay for *Chlamydia trachomatis* (APTIMA CT Assay) is a second generation NAAT that utilizes target capture, Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (HPA) technologies to streamline specimen processing, amplify target rRNA, and detect amplicon, respectively. Recent studies comparing performance and specimen inhibition of various amplification systems have demonstrated the benefits of target capture, TMA, and HPA (7, 11).

According to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* 2002 Screening Guidelines, CDC recommends a number of options for follow-up on a positive screening test "if a low positive predictive value can be expected or if a false-positive result would have serious psychosocial or legal consequences" (5). One of these options for additional testing can be a different FDA-cleared nucleic acid amplification test that targets a different nucleic acid sequence than the initial test. The APTIMA CT Assay targets different nucleic acid sequences than those targeted by other *C. trachomatis* NAATs, including the APTIMA Combo 2 Assay.

Principles of the Procedure

The APTIMA CT Assay combines the technologies of target capture, TMA, and HPA.

Swab or urine specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the APTIMA CT Assay is performed in the laboratory, the target rRNA molecule is isolated from the urine and swab samples by the use of a capture oligomer in a method called target capture; magnetic micro particles are another key feature of target capture. The capture oligomer contains a sequence complementary to a specific region of the target molecule as well as a string of deoxyadenosine residues. During the hybridization step, the sequence specific region of the capture oligomer binds to a specific region of the target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The micro particles, including the captured target molecule bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

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

Reagents

Reagents for the APTIMA Assay for CT are provided below. Reagent Identification Symbols are also listed next to the reagent name.

1 Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The vaginal swab specimen collection kit is not for home use.

Materials Provided

GEN-PROBE APTIMA® Assay for *Chlamydia trachomatis*
Catalog number: 1088

The GEN-PROBE APTIMA Assay for *Chlamydia trachomatis* provides the following reagents:

Refrigerated Box (2°C to 8°C).

Refrigerated Storage Tray (2°C to 8°C):

Symbol	Component	Quantity	Description
E	APTIMA Enzyme Reagent	1 x 100 tests	Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.
A	APTIMA Amplification Reagent CT	1 x 100 tests	Nucleic acids dried in buffered solution containing < 5% bulking agent.
P	APTIMA Probe Reagent CT	1 x 100 tests	Non-infectious chemiluminescent DNA probes (< 500 ng/vial) dried in succinate buffered solution containing < 5% detergent.
TCR-B	APTIMA Target Capture Reagent B	1 x 0.35 mL	Non-infectious nucleic acid in a buffered solution containing < 5% detergent.
PGC/ NCT	APTIMA CONTROL + GC PGC / CONTROL - CT NCT	3 x 1.7 mL	Non-infectious *N. gonorrhoeae* nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 50 *N. gonorrhoeae* cells (250 fg/assay*).
PCT/ NGC	APTIMA CONTROL + CT PCT / CONTROL - GC NGC	3 x 1.7 mL	Non-infectious *C. trachomatis* nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 1 *C. trachomatis* IFU (5 fg/assay*).

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

Storage Tray (2°C to 30°C)

AR	APTIMA Amplification Reconstitution Solution CT	1 x 9.3 mL	Aqueous solution containing preservatives.
ER	APTIMA Enzyme Reconstitution Solution	1 x 3.3 mL	HEPES buffered solution containing a surfactant and glycerol.
S	APTIMA Selection Reagent	1 x 3.1 mL	600 mM borate buffered solution containing surfactant.
PR	APTIMA Probe Reconstitution Solution CT	1 x 12.4 mL	*Succinate buffered solution containing < 5% detergent.*

Non-Refrigerated Box (15°C to 30°C)

Symbol	Component	Quantity	Description
TCR	APTIMA Target Capture Reagent CT	1 x 22 mL	Buffered salt solution containing solid phase (< 0.5 mg/ml) and capture oligomers.
W	APTIMA Wash Solution	1 x 402 mL	10 mM HEPES buffered solution containing < 2% detergent.
DF	APTIMA Buffer for Deactivation Fluid	1 X 402 mL	800 mM bicarbonate buffered solution.
O	APTIMA Oil Reagent	1 x 24.6 mL	Silicone oil

Also included in the kit are the following:

	Reconstitution Collars	3 each	
	Sealing Cards	1 package	

Materials Required But Not Provided

APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens

APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens

APTIMA Vaginal Swab Specimen Collection Kit

GEN-PROBE LEADER HC+ Luminometer

GEN-PROBE Target Capture System (TCS)

APTIMA Auto Detect Kit

2 Repeat pipettors

Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL)

Either:

2 Multi-tube vortex mixers

3 Circulating water baths (62°C± 1°C, 42°C ± 1°C, 62°C ± 1°C)

3 Water bath inserts

Or: 2 SB100 Dry Heat Bath/Vortexers (Additional SB100 Dry Heat Bath/Vortexers may be required as test volume increases)

Micropipettor: 20 µL to 200 µL

Tips, Pipetman P1000 Style (Special diameter tip - only available from Gen-Probe)

Tips, 1000 µL conductive, liquid sensing, TECAN 10612513

Pipette tips 20 µL to 200 µL

Household bleach (sodium hypochlorite solution)

Large-capped plastic container

Standard urine collection containers, without preservatives

Materials Available from Gen-Probe

Note: Gen-Probe catalog numbers are listed in parentheses.

APTIMA® Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens (Cat. No. 1041)

APTIMA® Urine Specimen Collection Kit for Male and Female Urine Specimens (Cat. No. 1040)

APTIMA® Vaginal Swab Specimen Collection Kit (Cat. No. 1162)

GEN-PROBE® LEADER® HC+ Luminometer (Cat. No. 4747-01)

GEN-PROBE® Target Capture System (TCS) (Cat. No. 4555)

APTIMA® Auto Detect Kit (Cat. No. 1048)

APTIMA® Controls Kit (Cat. No. 1110)

STD Proficiency Panel (Cat. No. 2325)

Multi-tube vortex mixer (Cat. No. 2160)

Circulating water bath (Cat. No. 4586)

Water bath insert (Cat. No. 4627)

SB100® Dry Heat Bath/Vortexer (Cat. No. 5524)

Pipettor, 1000 µL Rainin (Cat. No. 4216)

eppendorf Repeat Pipettor 20 µL to 200 µL (Cat. No. 5726)
Tips, Pipetman P1000 Style (Cat. No. 5049)

Ten Tube Units (TTU) (Cat. No. TU0022)

Ten Tip Cassettes (TTC) (Cat. No. 4578)

Non-penetrable caps (Cat. No. 3036A)

Penetrable caps (Cat. No. 5668)

SysCheck (Cat. No. 1078-01)

Warnings and Precautions

A. For *in vitro* diagnostic use.

Laboratory Related

B. Use only supplied or specified disposable laboratory ware.

C. 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.

D. **Warning: Irritants, Corrosives.** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash the effected area with water. If these fluid spill, dilute the spill with water before wiping it dry.

E. Work surfaces, pipettes, and other equipment must be regularly decontaminated with a 1:1 dilution of bleach (1 part bleach, 1 part water). Refer to *Procedural Notes* on page 7 and *Equipment Preparation* on page 4.

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

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

Specimen Related

H. For the collection of endocervical and male urethral swab specimens, use only the APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens. For urine specimen collection, use only the APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens. For clinician- and patient-collected vaginal swab specimens use only the APTIMA Vaginal Swab Specimen Collection Kit.

I. After urine addition, the liquid level in the urine transport tube must fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.

J. 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.

K. 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.

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

M. If the lab receives a swab specimen transport tube with no swab, two swabs, or a swab not supplied by Gen-Probe, the specimen must be rejected.

Assay Related

N. Do not use this kit after its expiration date. **Do not** interchange, mix, or combine reagents from kits with different lot numbers.

O. Tips with hydrophobic plugs must be used. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the Target Capture and Amplification steps, and one for use in the HPA steps. Two micropipettors must be dedicated for use in this assay: one for use in specimen transfer and one for use in reagent preparation. All pipettors must be cleaned regularly as described in *Procedural Notes* on page 7.

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

Q. Adequate mixing is necessary to achieve accurate assay results. For complete details, see *Procedural Notes* on page 7.

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

S. Upon piercing, liquid can discharge from APTIMA transport tube caps under certain conditions. Follow instructions in Target Capture, Rack Setup, step 3, on page 5 to prevent this occurrence.

T. Assay reproducibility was established using spiked swab transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined.

Storage and Handling Requirements

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

APTIMA Enzyme Reagent

APTIMA Amplification Reagent CT

APTIMA Probe Reagent CT

APTIMA Target Capture Reagent B

APTIMA CONTROL + GC PGC / CONTROL - CT NCT
*Neisseria gonorrhoeae (*GC) positive control / *Chlamydia trachomatis* (CT) negative control

APTIMA CONTROL + CT PCT / CONTROL - GC NGC
Chlamydia trachomatis (CT) positive control / *Neisseria gonorrhoeae* (GC) negative control)

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

APTIMA Amplification Reconstitution Solution CT

APTIMA Enzyme Reconstitution Solution

APTIMA Selection Reagent

APTIMA Probe Reconstitution Solution CT

C. The following reagents are stable when stored at 15°C to 30°C:

APTIMA Wash Solution

APTIMA Buffer for Deactivation Fluid

APTIMA Oil Reagent

D. The Target Capture Reagent CT is stable when stored at room temperature (15°C to 30°C). **Do not** store at temperatures below 15°C.

E. Once combined, the Target Capture Reagent CT plus the Target Capture Reagent B is stable for 60 days when stored at 15°C to 30°C.

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

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

H. Upon Warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not effect 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).

I. **Do not freeze the reagents**.

Specimen Collection and Storage

The APTIMA CT Assay is designed to detect the presence of CT in clinician-collected endocervical, vaginal and male urethral swab specimens, patient-collected vaginal swab specimens, and female and male urine specimens. Only the swabs and the specimen transport tubes contained in the APTIMA Vaginal Swab Collection Kit and APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens can be used to collect patient swab specimens. Performance has not been established with other products.

Swab specimens must be transported to the laboratory in the swab specimen transport medium and tube. Swab specimens must be transported to the laboratory at 2°C to 30°C and tested within 60 days of collection.

Urine specimens can be transported to the laboratory at 2°C to 30°C in either the primary collection device (urine cup) or in the urine specimen transport tube. Urine specimens must be transferred into the APTIMA urine specimen transport tube within 24 hours of collection and before being assayed. After transfer, urine specimens can be stored at 2°C to 30°C for up to 30 days after collection.

Specimen collection instructions for endocervical swab, vaginal swab, male urethral swab, and urine specimens are provided in each respective GEN-PROBE APTIMA specimen collection kit.

A. Specimen transport and storage before testing:
   1. Swab specimens:
      a. After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested. Specimens must be assayed with the APTIMA CT Assay within 60 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 90 days after collection.
   2. Urine Specimens:
      a. 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 CT Assay within 30 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 90 days after collection.
      b. 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. 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 or foil barrier.
   3. If assayed samples need to be frozen or shipped, remove penetrable cap and place new non-penetrable or penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 RCF (Relative Centrifugal Force) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination**.

Note: Federal requirements for packaging must be met when specimens are transported by common land and air carriers. Refer to 42 CFR, Part 72. The most current requirements may be obtained from the Centers for Disease Control and Prevention Office of Health and Safety (CDC) in Atlanta, Georgia at 1-800-311-3435.

Test Procedure

A. Equipment Preparation
   1. Adjust one water bath to 62°C ± 1°C (for target capture, and primer annealing), a second water bath to 42°C ± 1°C (for amplification), and a third water bath to 62°C ± 1°C (for HPA). If using the SB100 Dry Heat Bath/Vortexer, refer to the SB100 Application Sheet.
   2. Prior to starting the assay, wipe down work surfaces and pipettors with household bleach diluted 1:1 with water (one part bleach, one part water). Allow the bleach to contact surfaces and pipettors for at least one minute, then follow with a water rinse. Do not allow the bleach to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed, absorbent laboratory bench covers.
   3. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with APTIMA Wash Solution and the aspirator is connected to the vacuum pump. (Refer to the *Target Capture System Operator's Manual*.)

B. Reagent Reconstitution

   Reagent Reconstitution should be performed prior to beginning specimen transfer.
   1. To reconstitute the APTIMA Enzyme, Amplification CT, and Probe CT Reagents:
      a. Pair the appropriate reconstitution solution with the dried reagent. The labels have been color coded so that they can be paired correctly.
      b. Open the dried reagent and firmly insert the notched end of the reconstitution collar into the glass vial (Figure1, Step 1).
      c. Open the reconstitution solution (save the cap) and, while holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle (Figure 1, Step 2).
      d. Invert the assembly, allow the solution to drain into the glass container (Figure 1, Step 3), then gently swirl the solution within the container (Figure1, Step 4). Invert the assembly and tilt it at a 45° angle (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.
      e. Remove the reconstitution collar and the glass vial (Figure1, Step 6).
      f. Discard both the reconstitution collar and glass vial (Figure1, Step 7).
      g. Recap the plastic bottle and peel away the top label on the reconstituted reagent. Record required information on the remaining bottle label (Figure 1, Step 8).

Figure 1. Reconstitution process

h. Discard reconstituted reagent after 60 days or by the expiration date, whichever comes first.

2. If previously reconstituted Probe CT, Amplification CT, and Enzyme Reagents are being used, allow them to reach room temperature (15° to 30°C) prior to the start of the assay. If Probe Reagent has a precipitate and it does not go back into solution at room temperature, heat the reagent solution at 62°C for 1 to 2 minutes. After this heat step, the Probe Reconstitution Solution may be used even if residual precipitate remains. After resuspension, mix the vial by gentle inversion.

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

3. Prepare a solution of Target Capture Reagent CT and Target Capture Reagent B (TCR CT plus TCR-B) as follows:

   a. Determine the number of reactions to be performed (specimens plus controls).

   b. Calculate the volumes of Target Capture Reagent CT (TCR CT) and Target Capture Reagent B (TCR-B) as follows:

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

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

TCR CT plus TCR-B Preparation (Example)

Number of Reactions	TCR CT	TCR-B
25 + 5	3.0 mL	0.03 mL (30 µL)
75 + 5	8.0 mL	0.08 mL (80 µL)
100 + 5	10. 5 mL	0.105 mL (105 µL)

   c. Transfer the calculated volume of TCR CT to an appropriately sized, dedicated, clean, dry container and, using a micropipettor, add the calculated volume of TCR-B into the TCR CT.

   d. Thoroughly mix the solution by swirling.

   e. The TCR CT and TCR-B solution is stable for 60 days when stored at 15º to 30ºC. Do not refrigerate.

C. Target Capture

The repeat pipettor used in target capture and amplification should be dedicated for use in these steps only. See *Materials Provided* on page 2.

Note: If the lab receives a swab specimen transport tube with no swab, two swabs, or a swab not supplied by Gen-Probe, the specimen must be rejected. After urine addition, the liquid level in the urine specimen transport tube must fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.

Rack Setup

1. Allow the urine and swab specimens to reach room temperature prior to processing.
2. **Do not vortex specimens.**
3. Inspect transport tubes before piercing them:

   a. If a transport 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 transport 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 is not between the two black indicator lines on the urine transport tube label, the specimen must be rejected. Do not pierce an overfilled tube.

   d. If a urine specimen contains precipitates, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, ensure that the precipitate does not prevent delivery of the specimen.

   Note: Failure to follow steps 3a-c may result in liquid discharge from the transport tube cap.

4. In the Ten Tube Unit (TTU) rack, place enough TTUs to accommodate the controls and specimens.
5. If a worklist is desired, create the worklist at this point. For instructions on creating a worklist, refer to the *APTIMA Assay Software Operator's Manual*.
6. Thoroughly mix the TCR CT plus TCR-B reagent. Using the repeat pipettor, add 100 µL into each reaction tube.
7. **The first tube of the assay must contain the negative control, and the second tube must contain the positive control.** The negative control label for the APTIMA CT Assay is blue-green. The label text identifies the negative control as "CONTROL + GC PGC / CONTROL − CT NCT" The positive control label for the APTIMA CT Assay is pink. The label text identifies the positive control as "CONTROL + CT PCT / CONTROL − GC NGC".

   Hold the negative control tube in one hand or keep it in a rack. Using a micropipettor, pierce the cap, taking care not to drive the tip into the bottom of the tube. Add 400 µL of the negative control to the first reaction tube. In the same manner, add 400 µL of the positive control to the second reaction tube.
8. Continue the rack setup procedure by adding 400 µL of each specimen to the remaining TTU tubes. Use a new pipette tip for each specimen and control. The acceptable volume of control or specimen added to the TTU is 400 µL ± 100 µL. See *Control and Specimen Pipetting* in *Procedural Notes* on page 7.
9. If specimens with standard caps (non-penetrable caps) are to be tested, they must be centrifuged for 5 minutes at 420 RCF (Relative Centrifugal Force) to bring all of the liquid down to the bottom of the tube before uncapping. **Avoid splashing and cross-contamination.**

Target Capture

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

10. Cover the TTUs with sealing cards and shake the rack gently by hand. **Do not vortex.** Incubate the rack at 62°C ± 1°C in a water bath for 30 ± 5 minutes.
11. Remove the rack from the water bath and blot the bottoms of the tubes dry on absorbent material.
12. Ensure the sealing cards are firmly seated. If necessary, replace them with new sealing cards and seal the TTUs tightly.

13. Vortex the rack for 60 seconds on the multi-tube vortex mixer. See *Vortexing* on page 7 in *Procedural Notes*. Begin vortexing within 2 minutes of removal of the rack from the water bath.
14. Without removing the sealing cards, incubate the rack at room temperature for 30 ± 5 minutes.
15. Place the rack on the TCS magnetic base for 5 to 10 minutes.
16. Prime the dispense station pump lines by pumping APTIMA Wash Solution through the dispense manifold. Pump enough liquid through the system so that there are no air bubbles in the line and all ten nozzles are delivering a steady stream of liquid.
17. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the aspiration manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification.* It may take 15 seconds to achieve this reading. Reconnect the manifold, and ensure that the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed.

    *See the Target Capture System Vacuum Specifications Sheet located at the back of the *Target Capture System Operator's Manual* or contact Technical Support.
18. Firmly attach the aspiration manifold to the first set of tips. Aspirate all liquid by lowering the tips into the first TTU until the tips come into brief contact with the bottoms of the tubes. Do not hold the tips in contact with the bottoms of the tubes.
19. After the aspiration is complete, eject the tips into their original tip cassette. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each specimen.
20. Place the dispense manifold over each TTU and, using the dispense station pump, deliver 1.0 mL of APTIMA Wash Solution into each tube of the TTU.
21. Cover the tubes with a sealing card and remove the rack from the TCS. Vortex the rack once on the multi-tube vortex mixer. See *Vortexing* in *Procedural Notes* on page 7.
22. Place the rack on the TCS magnetic base for 5 to 10 minutes.
23. Aspirate all liquid as in Steps 18 and 19.
24. After the final aspiration, remove the rack from the TCS base and visually inspect the tubes to ensure that all liquid has been aspirated. If any liquid is visible, place the rack back onto the TCS base for 2 minutes and repeat the aspiration for that TTU using the same tips used previously for each specimen. If any magnetic particle pellet is visible after aspiration is completed, the tube may be accepted. If no pellet is visible, the specimen should be retested. If the same specimen did not contain a magnetic particle pellet at this step in a subsequent run, this may indicate a specimen-specific problem. Re-collection of the specimen is recommended in this situation.

D. Amplification

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

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

E. Hybridization Protection Assay (HPA)

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

The repeat pipettor used in hybridization and selection should be dedicated for these steps only. See *Materials Provided* on page 2.

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

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

        Note: This temperature range is critical for assay performance.
    b. For use of the LEADER HC+ Luminometer and the APTIMA Assay Software refer to the *LEADER HC+ Luminometer Operator's Manual* and the *APTIMA Assay Software Operator's Manual.*
    c. Prepare the LEADER HC+ Luminometer by placing one empty TTU in cassette position number one and performing the **Wash** protocol.
    d. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the tests.
    e. Load the TTUs into the luminometer.
    f. Log on to the computer. Click on **New Run** choose **APTIMA CT Assay Protocol** and enter the number of tubes (controls and specimens). Click **Next** to begin the run.

        Note: The run must be completed within 2 hours of the end of the selection step incubation.
    g. Prepare a buffered bleach deactivation solution by mixing equal volumes of household bleach and APTIMA Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. This buffered bleach solution is stable for four weeks at room temperature.
    h. After removing the used TTUs from the luminometer, place the TTUs into the container with the buffered bleach solution. Allow the TTUs to sit in the container for 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

F. Lab Contamination Monitoring Protocol

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 the Endocervical and Male Urethral Swab Specimens:

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

Interpretation

If the results are CT positive or equivocal, the surface may be contaminated and should be decontaminated by treating with bleach as recommended in *Equipment Preparation* on page 4. See *Test Interpretation - QC/Patient Results* on page 8.

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

Procedural Notes

A. Controls

To work properly with the APTIMA Assay Software, the positive control for GC, which is labeled "CONTROL + GC PGC / CONTROL - CT NCT" must be in the first position of the first TTU. The positive control for CT, which is labeled "CONTROL + CT PCT / CONTROL - GC NGC" must be in the second position of the first TTU. Placement in the wrong position will cause the run to fail. Any additional controls must be entered as patient specimens and monitored by the operator for acceptability. The positive control for GC, which is labeled "CONTROL + GC PGC / CONTROL - CT NCT" serves as the negative control for the APTIMA CT Assay.

B. Control and Specimen Pipetting

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

C. Reagents

Probe Reconstitution Solution may precipitate during storage. If this occurs, heat the Probe Reconstitution Solution at 62°C for 1 to 2 minutes. After this heat step, the Probe Reconstitution Solution may be used even if residual precipitate remains. After resuspension, mix the vial by gentle inversion.

D. Temperature

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

E. Time

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

F. Glove Powder

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

G. Vortexing

Proper vortexing is important to the successful performance of the APTIMA CT Assay. Vortexing is the manipulation by an external energy source of a solution to produce a uniform suspension. If an adequate vortexing motion is achieved, the suspension rotates in a circular motion at a rate capable of raising the solution to a height within the upper half of the tube. This manipulation is maintained for specified periods of time. To vortex reactions, set the multi-tube vortex mixer speed to the lowest setting, secure the rack, and turn on power. Slowly increase speed until the liquid goes halfway up the tube. Vortex for 10 seconds, the indicated amount of time, or until the color is uniform. Then, turn speed to lowest setting before turning off the multi-tube vortex mixer and removing the rack. The reaction mixtures should never touch the sealing cards.

H. Water Baths

1. The level of the water in the water baths must be maintained at 2.5" to 3.5" deep as measured from the supporting metal tray (on the bottom of the water bath) to the surface of the water. This will ensure proper heat transfer.
2. To avoid cross-contamination, water baths should be dedicated to a specific assay step.

I. Decontamination

1. Surfaces and Pipettors
   Laboratory bench surfaces and pipettors must be decontaminated regularly with household bleach diluted 1:1 with water, (1 part bleach, 1 part water). Allow bleach to contact surfaces for at least 1 minute and then follow with a water rinse. **Do not allow the bleach to dry**. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment with water to avoid pitting.
2. TCS Manifold
   Disconnect the aspiration manifold by removing the tube from the tube attachment. Submerge the manifold in household bleach diluted 1:1 with water, ensuring that the handles and pipette tip nozzles are covered by the bleach solution. Keep the manifold submerged for 10 minutes. Longer exposure will damage the manifold. Rinse the manifold thoroughly with water and then dry completely with paper towels. Ensure that the area under the ejector plate is dry.
3. TCS Waste Container
   Disconnect the waste bottle from the unit and pour the waste into a sink. Pour 400 µL of bleach into the bottle. Leaving the bleach in the bottle, reconnect the bottle to the unit. Reconnect the manifold and run the pump for 3 minutes to complete the drying process.
4. TCS Unit
   Wipe the surfaces of the TCS unit and surface of the Wash Buffer ejector tips with paper towels moistened with bleach diluted 1:1 with water. Follow the bleach step with a water rinse and then dry the unit completely with paper towels.
5. Racks
   Submerge the racks in household bleach diluted 1:1 with water, ensuring that they are covered by the bleach solution. Keep the

racks submerged for 10 minutes. Longer exposure will damage the racks. Rinse the racks thoroughly with water, then dry them completely with paper towels.

J. Assay Contamination

1. The introduction of contaminating materials may occur if sufficient care is not taken during the assay protocol.
2. TTUs must be decontaminated with buffered bleach as described in the Detection portion of the assay protocol. Do not reuse the TTUs.
3. Perform regular decontamination of equipment and work surfaces as described above in *Procedural Notes*, *Decontamination*.
4. As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. It is recommended that operators use powderless gloves.

K. Troubleshooting

1. Low positive control values may be caused by incorrect temperatures during various steps in the assay or by allowing the selection time in the selection step to go longer than the recommended time.
2. High backgrounds may occur if the selection time in the selection step is shortened, the selection temperature is not correct, or insufficient mixing occurs after the addition of the Selection Reagent.
3. If the APTIMA positive control for GC, which is labeled "CONTROL + GC PGC / CONTROL - CT NCT", is positive or equivocal for CT, see *Assay Contamination* on page 8 and/or *Procedural Notes* on page 7.

Test Interpretation - QC/Patient Results

A. Test Interpretation

Assay test results are automatically interpreted by the APTIMA Assay Software using the CT protocol. A test result may be negative, equivocal, positive, or invalid as determined by total RLU in the detection step (see below). A test result may be invalid due to RLU values outside the normal expected ranges. Initial equivocal and invalid test results should be repeated.

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

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

[1]According to CDC guidelines, "consideration should be given to routine additional testing for persons with positive CT or GC screening tests when risk-factor information or actual surveys indicate that the prevalence is low, resulting in a lower PPV (e.g., <90%)." Refer to CDC guidelines for details on additional testing and patient management after a positive screening test (5).

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

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

B. Quality Control Results and Acceptability

Controls must be run with each assay. The APTIMA Positive Control for GC, which is labeled, "CONTROL + GC PGC / CONTROL - CT NCT" and the APTIMA Positive Control for CT, which is labeled, "CONTROL + CT PCT / CONTROL - GC NGC" act as controls for the **target capture**, **amplification**, and **detection** steps of the assay. In accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations, additional controls for cell lysis and RNA stabilization may be included. The positive control for GC, which is labeled, "CONTROL + GC PGC / CONTROL - CT NCT" contains non-infectious GC rRNA. If desired, additional controls can be ordered as a kit. See *Materials Available from Gen-Probe* on page 2. Correct preparation of specimens is confirmed visually by the presence of a single GEN-PROBE collection swab in a swab specimen transport tube, or a final volume of urine in between the black fill lines of a urine specimen transport tube.

The APTIMA Assay Controls must produce the following test results:

Control	Total RLU (x1000)	CT Result
CONTROL + GC PGC / CONTROL - CT NCT	0* and < 50	Negative
CONTROL + CT PCT / CONTROL - GC NGC	≥ 100 and < 12,000	Positive

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

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. If the Run Status is FAIL, all test results in the same run are invalid and must not be reported.

Each laboratory should implement appropriate control procedures to satisfy the requirements of CLIA regulations (section 493.1256).

See *Troubleshooting* on page 8, or call Gen-Probe Technical Support for help with out-of-range controls.

C. Specimen Preparation Control (optional)

The APTIMA Positive Control for GC, which is labeled, "CONTROL + GC PGC / CONTROL - CT NCT" and the APTIMA Positive Control for CT which is labeled, "CONTROL + CT PCT / CONTROL - GC NGC" act as controls for the **target capture**, **amplification**, and **detection** steps of the assay and must be included in each assay run. If desired, controls for cell lysis and RNA stabilization can be tested in accordance with the requirements of appropriate accrediting organizations or individual laboratory procedures. Known positive specimens can serve as controls by being prepared and tested in conjunction with unknown specimens. Specimens used as preparation controls must be stored, handled, and tested according to the package insert. Specimen preparation controls should be interpreted in the same manner as described for patient test specimens. See *Test Interpretation - QC/Patient Results* on page 8.

D. Patient Test Results

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

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

b. Retest results

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

*Low RLU Positive specimen results are included in this category. See *Test Interpretation* above.

Notes

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

Limitations

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

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

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

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

E. The APTIMA CT Assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications. For those patients for whom a false positive result may have adverse psycho-social impact, CDC recommends retesting by a method using an alternate technology (5).

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 package insert of the appropriate GEN-PROBE APTIMA specimen collection kit.

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

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

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

J. The APTIMA CT Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the number of organisms in a specimen.

K. Performance characteristics for detecting CT are derived from high prevalence populations. Positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.

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

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

N. APTIMA CT Assay has not been validated for use with vaginal swab specimens collected by patients at home.

O. The performance of the vaginal swab specimen has not been evaluated in pregnant women.

P. The performance of the vaginal swab specimen has not been evaluated in teenage women less than 16 years of age.

Clinical Study Results

The performance characteristics of the APTIMA CT Assay were established in a multi-center clinical investigation conducted in North America. In this clinical investigation, two studies were conducted. One, the clinical specimen study, established the sensitivity, specificity, and predictive values of the APTIMA CT Assay using clinician-collected endocervical, vaginal, and male urethral swab specimens, patient-collected vaginal swab specimens, and male and female urine specimens. The second study evaluated the precision of the APTIMA CT Assay when performed according to NCCLS Guidelines (16).

Expected Values

Prevalence

The prevalence of CT in patient populations depends on risk factors such as age, gender, the presence of symptoms, the type of clinic, and the test method. A summary of the prevalence of CT, by specimen type as determined by the APTIMA CT Assay is shown in Table 1 by clinical site and overall.

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

The estimated positive and negative predictive values (PPV and NPV) for different hypothetical prevalence rates using the APTIMA CT Assay are shown in Table 2. These calculations are based on hypothetical prevalence rates and the overall sensitivity and specificity estimated from the patient infected status. The overall sensitivity and specificity for CT was 97.0% and 96.4%, respectively (Table 2). The actual PPV and NPV for clinician-collected endocervical, vaginal and male urethral swab, patient-collect vaginal swab, and male and female urine specimens are shown in Table 5 for each clinical site and overall.

Table 1. Prevalence of *C. trachomatis* by Clinical Site and Overall as Determined by APTIMA CT Assay Results

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

MS = Male Urethral Swab; **MU** = Male Urine; **FS** = Female Endocervical Swab; **FU** = Female Urine; **PVS** = Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab.

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

Hypothetical Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
2	97.0	96.4	35.4	99.9
5	97.0	96.4	58.6	99.8
10	97.0	96.4	74.9	99.7
15	97.0	96.4	82.6	99.4
20	97.0	96.4	87.0	99.2
25	97.0	96.4	90.0	99.0
30	97.0	96.4	92.0	98.7

APTIMA CT Assay RLU Distribution

Figure 2 shows the RLU distribution for the APTIMA CT Assay for all specimen types in the clinical study. Table 3 summarizes the RLU distribution for the total positive and total negative results, as well as the false positive and false negative results for each specimen type relative to infected patient status. Across certain specimen types, there is a trend toward an increasing proportion of true positives as the RLU values increase.

Figure 2 Frequency of RLU Distribution for the APTIMA CT Assay

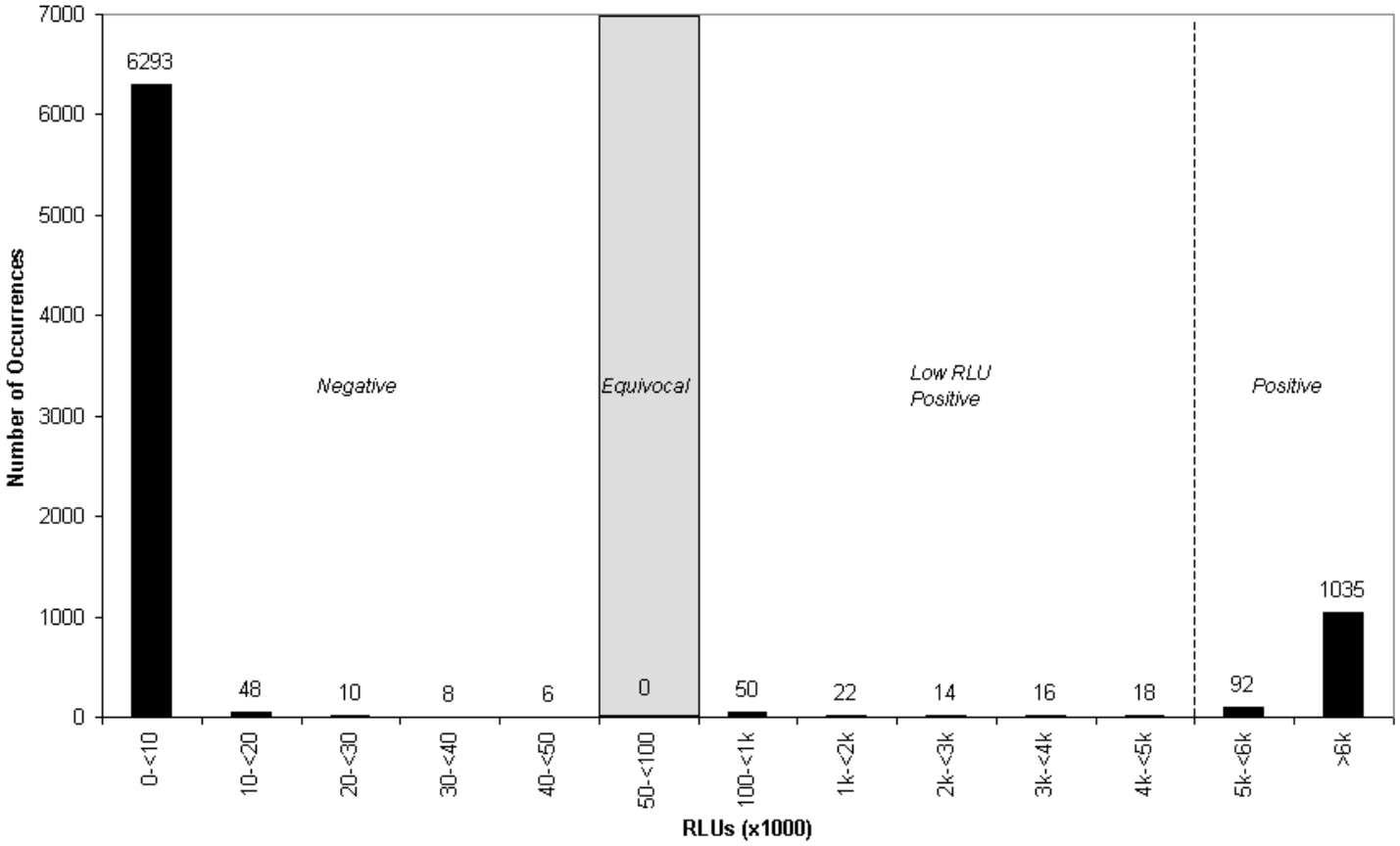

Table 3. APTIMA CT Assay RLU Distribution

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

CVS = Clinician-Collected Vaginal Swab; **PVS** = Asymptomatic Patient-Collected Vaginal Swab; **FS** = Female Endocervical Swab; **MS** = Male Urethral Swab; **FU** = Female Urine; **MU** = Male Urine.

Shaded column denotes equivocal zone.

Clinical Performance Characteristics

Clinical Specimen Study

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

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

All performance calculations were based on the total number of APTIMA CT Assay results for endocervical, vaginal and male urethral swab, and male and female urine specimens compared to a patient infected status algorithm for each gender. In the algorithm, the designation of a subject as being infected or not infected with CT was based on endocervical swab and urine specimen results from the commercially-available APTIMA Combo 2 Assay and the other commercially-available NAAT. Subjects were considered infected with CT if two of the four endocervical swab and urine

specimens tested positive in the APTIMA Combo 2 Assay and the other reference NAAT (one specimen testing positive in each NAAT). Subjects were considered non-infected if less than two reference NAAT results were positive.

A total of 8,406 APTIMA CT Assay results were used to calculate sensitivity and specificity. Sensitivity and specificity for CT by gender, specimen type and symptom status are presented in Table 4. Table 5 shows the APTIMA CT Assay sensitivity, specificity, and predictive values compared to patient infected status for each clinical site and overall. Tables 6a-6d summarize the number of results from symptomatic and asymptomatic subjects designated as infected or non-infected with CT according to the patient infected status algorithm.

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

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

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

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

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

Table 5. Sensitivity, Specificity, and Predictive Values of the APTIMA CT Assay Relative to Patient Infected Status by Clinical Site and Overall

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

Table 5. Sensitivity, Specificity, and Predictive Values of the APTIMA CT Assay Relative to Patient Infected Status by Clinical Site and Overall

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

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

Table 6a. Male Urethral Swab and Urine Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

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

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

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

Table 6b. Female Endocervical Swab and Urine Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

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

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

FS = Female Endocervical Swab; **FU** = Female Urine. **Symp.** = Symptomatic; **Asymp.** = Asymptomatic.

Table 6c. Asymptomatic Patient-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

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

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

FS = Female Endocervical Swab; **FU** = Female Urine; **CVS** = Clinician-Collected Vaginal Swab; **PVS** = Asymptomatic Patient-Collected Vaginal Swab.

Table 6d. Clinician-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

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

Table 6d. Clinician-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

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

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

FS = Female Endocervical Swab; **FU** = Female Urine; **CVS** = Clinician-Collected Vaginal Swab.

Symp. = Symptomatic; **Asymp.** = Asymptomatic.

The distribution of the RLUs for the APTIMA Positive Control for GC (Negative Control for CT) and the APTIMA Positive Control for CT (Negative Control for GC) from all the APTIMA CT Assay runs performed during the clinical specimen study is presented in Table 7.

Table 7. Distribution of RLU of the APTIMA Controls

Control	Statistics	RLU (x1000)
Positive Control for GC/ Negative Control, CT	Maximum	26
	75th Percentile	1
	Median	0
	25th Percentile	0
	Minimum	0
Positive Control for CT/ Negative Control, GC	Maximum	8884
	75th Percentile	7440
	Median	7066
	25th Percentile	6621
	Minimum	988

Precision Study

APTIMA CT Assay precision (i.e., reproducibility) was evaluated at two external clinical sites and at Gen-Probe. APTIMA CT Assay precision was evaluated across three APTIMA CT Assay kit lots, three study sites, six operators and 108 APTIMA CT Assay runs. Two operators at each of the three testing sites performed a total of six APTIMA CT Assay runs per kit lot for a total of 36 runs per kit lot. Each run was composed of a 12-member precision panel containing 0 to 2,000 fg/assay of CT rRNA. Reproducibility was established using spiked swab transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Table 8 presents the precision RLU data in terms of Mean, Standard Deviation, Coefficient of Variation (CV), and percent agreement with expected results for calculations of inter-site, inter-lot, inter-operator, inter-run, and intra-run variability.

Table 8. APTIMA CT Assay Precision Data

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

SD = Standard Deviation; **CV(%)** = Percent Coefficient of Variation; **% Agrmt.** = Percent Agreement.

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and %CV is set to zero (16). **N/A** = not applicable for negative analyte.

Analytical Performance Characteristics

Analytical Sensitivity

C. trachomatis analytical sensitivity (limit of detection) was determined by directly comparing dilutions of CT organisms in cell culture and in the APTIMA CT assay. The analytical sensitivity claim for the assay is one Inclusion-Forming Unit (IFU) per assay (7.25 IFU/swab, 5 IFU/mL urine) for all 15 CT serovars. However, dilutions of less than one IFU/assay of all serovars tested positive.

Analytical Specificity

A total of 154 culture isolates were evaluated using the APTIMA CT Assay. These isolates included 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, parasites and viruses. All organisms except *C. psittaci, C. pneumoniae, U. urealyticum* and the viruses were tested at $1.0x10^6$ cells/assay in Kova-trol/Urine Transport Media and 60 organisms were tested in Swab Transport Media. *C. psittaci* VR601 was tested at $8x10^4$ cells/assay and *C. psittaci* VR125 was tested at $1x10^5$ cells/assay. *C. pneumoniae* was tested at $4x10^3$ cells/assay and U. urealyticum was tested at $6.7x10^6$ cells/assay. The viruses were tested as follows: (a) herpes simplex virus I: $2.5x10^4$ $TCID_{50}$/assay, (b) herpes simplex virus II: $6.0x10^4$ $TCID_{50}$/assay, (c) human papillomavirus 16: $2.9x10^6$ DNA copies/assay and (d) cytomegalovirus: $4.8x10^5$ cells/assay. The list of organisms tested is shown in Table 9.

Table 9. Analytical Specificity

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

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

Interfering Substances

The following substances commonly present in swab and/or urine specimens were tested in the assay:

Swab and PreservCyt liquid Pap	Urine
10% Blood	30% Blood
Contraceptive jelly	Urine analytes:
Spermicide	Protein
Moisturizer	Glucose
Hemorrhoidal anesthetic	Ketones
Body oil	Bilirubin
Powder	Nitrate
Anti-fungal cream	Urobilinogen
Vaginal lubricants	pH 4 (acidic)
Feminine spray	pH 9 (alkaline)
Leukocytes (1 x 10^6 cells/mL)	Leukocytes (1 x 10^6 cells/mL)
	Cellular debris
	Vitamins
	Minerals
	Acetaminophen
	Aspirin
	Ibuprofen

All were tested for potential assay interference in the absence and presence of CT at the estimated rRNA equivalent of 1cell/assay (5 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. No interference was observed with any of the tested substances. No inhibitors of amplification were observed in the APTIMA CT Assay.

Recovery

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

Swab and Urine Specimen Stability Studies

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

Data to support the recommended shipping and storage conditions for urine samples were generated with female and male negative urine samples. The urine samples were spiked with CT at a final concentration of 10 IFU per reaction. Two sets of the spiked urine samples were held at 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 CT at all timepoints. The two sets of UTM samples were also tested after 116 days of storage at -20°C and -70°C. All samples were positive for CT under both storage conditions.

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Refer also to the NCCLS document for the APTIMA CT Assay, accessible on the internet at: www.gen-probe.com.

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San Diego, CA 92121
Customer Service: (800) 523-5001
Technical Support: (888) 484-4747

www.gen-probe.com

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Authorized Representative
HCI, Dr. Roland Seidel GmbH
Health Care Industry Consultants
Attn. Dr. Roland Seidel
Paul-Ehrlich-Str.32-H8
D63322 Rodermark, Germany

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