GEN-PROBE®

APTIMA®

GEN-PROBE® APTIMA® Assay for *Neisseria gonorrhoeae*

For *in vitro* diagnostic use.

Intended Use

The APTIMA® Assay for *Neisseria gonorrhoeae* is a target amplification nucleic acid probe test that utilizes target capture for the *in vitro* qualitative detection of ribosomal RNA (rRNA) from *Neisseria gonorrhoeae* (GC) to aid in the diagnosis of gonococcal urogenital disease. The assay may be used to test the following specimens from symptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; and patient-collected female and male urine specimens. The assay may be used to test the following specimens from asymptomatic individuals: clinician-collected endocervical and vaginal swab specimens; and patient-collected vaginal swab specimens* and female and male urine specimens.

*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.

Summary and Explanation of the Test

Neisseria gonorrhoeae infections are one of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 361,705 new cases of *N. gonorrhoeae* infections were reported in 2001 (2).

N. gonorrhoeae is the causative agent of gonorrheal disease. *Neisseria* 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 Pelvic Inflammatory Disease (PID). PID 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) (8, 11).

Conventional diagnosis of *N. gonorrhoeae* infection requires isolation of the organism on selective media or the observation of diplococci in Gram stained smears (9). Culture methods can have good clinical sensitivity, but are highly dependent on proper specimen handling. Improper specimen storage and transport can result in the loss of organism viability and yield false negative results. In addition, poor sampling technique, toxic sampling materials, and the inhibition of growth by components of body secretions can also result in false negative results (3, 10). Commonly used non-culture methods for *N. gonorrhoeae* detection include direct DNA probe tests and nucleic acid amplification tests (NAATs).

First generation NAATs for *N. gonorrhoeae* have technological issues that have limited their performance. These issues include cumbersome specimen processing and specimen inhibition that can yield false negative results (6). The GEN-PROBE APTIMA Assay for *Neisseria gonorrhoeae* (APTIMA GC 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 (4, 7).

According to CT/GC Screening Guidelines, the Centers for Disease Control and Prevention (CDC) recommend that consideration should be given to routinely performing an additional test after a positive screening test if the positive predictive value is considered low. CDC recommends testing of the original specimen with another test that uses a different target, antigen, or phenotype and a different format (1).

Principles of the Procedure

The APTIMA GC Assay combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (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 GC 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 *N. gonorrhoeae* 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 GC Assay are provided below. Reagent Identification Symbols are also listed next to the reagent name.

APTIMA GC Assay Kit (2 boxes)

Refrigerated Box (2° to 8°C):

Refrigerated Storage Tray (2° to 8°C)

Symbol	Reagent Name	Quantity
E	APTIMA Enzyme Reagent *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 X 100 tests
A	APTIMA Amplification Reagent GC *Nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 X 100 tests
P	APTIMA Probe Reagent GC *Non-infectious chemiluminescent DNA probes (< 500 ng/vial) dried in succinate buffered solution containing < 5% detergent.*	1 X 100 tests
TCR-B	APTIMA Target Capture Reagent B *Non-infectious nucleic acid in a buffered solution containing < 5% detergent.*	1 X 0.35 mL
PGC/NCT	APTIMA Positive Control, GC/Negative Control, CT *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 *CFU (250 fg/assay*).*	3 X 1.7 mL
PCT/NGC	APTIMA Positive Control, CT/Negative Control, GC *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*).*	3 X 1.7 mL

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

Storage Tray (2° to 30°C)

AR	APTIMA Amplification Reconstitution Solution GC *Aqueous solution containing preservatives.*	1 X 9.3 mL
ER	APTIMA Enzyme Reconstitution Solution *HEPES buffered solution containing a surfactant and glycerol.*	1 X 3.3 mL
S	APTIMA Selection Reagent *600 mM borate buffered solution containing surfactant.*	1 X 31 mL
PR	APTIMA Probe Reconstitution Solution GC *Succinate buffered solution containing < 5% detergent.*	1 X 12.4 mL

Non-Refrigerated Box (15° to 30°C):

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

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 affected area with water. If these fluids 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 areas.

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 observing 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. 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. Tips with hydrophobic plugs must be used for specimen transfer. 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, to prevent this occurrence.

T. Assay reproducibility was established using swab transport medium spiked 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° to 8°C:
APTIMA Enzyme Reagent
APTIMA Amplification Reagent GC
APTIMA Probe Reagent GC
APTIMA Target Capture Reagent B
APTIMA Positive Control, GC / Negative Control, CT
APTIMA Positive Control, CT/Negative Control, GC

B. The following reagents are stable when stored at 2° to 30°C:
APTIMA Amplification Reconstitution Solution GC
APTIMA Enzyme Reconstitution Solution
APTIMA Selection Reagent
APTIMA Probe Reconstitution Solution GC

C. The following reagents are stable when stored at 15° to 30°C:
APTIMA Wash Solution
APTIMA Buffer for Deactivation Fluid
APTIMA Oil Reagent

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

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

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

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

H. **Do not freeze the reagents**.

Materials Provided

The APTIMA GC Assay provides the following reagents:

Catalog number: 1091	**100 tests**
Refrigerated Box (2° to 8°C):	
Refrigerated Storage Tray (2° to 8°C)	
APTIMA Enzyme Reagent	1 X 100 tests
APTIMA Amplification Reagent GC	1 X 100 tests
APTIMA Probe Reagent GC	1 X 100 tests
APTIMA Target Capture Reagent B	1 X 0.35 mL
APTIMA Positive Control, GC / Negative Control, CT	3 X 1.7 mL
APTIMA Positive Control, CT/Negative Control, GC	3 X 1.7 mL
Storage Tray (2° to 30°C)	
APTIMA Amplification Reconstitution Solution GC	1 X 9.3 mL
APTIMA Enzyme Reconstitution Solution	1 X 3.3 mL
APTIMA Selection Reagent	1 X 31 mL
APTIMA Probe Reconstitution Solution GC	1 X 12.4 mL
Reconstitution Collars	3 each
Sealing Cards	1 package
Non-Refrigerated Box (15° to 30°C):	
APTIMA Target Capture Reagent GC	1 X 22 mL
APTIMA Wash Solution	1 X 402 mL
APTIMA Buffer for Deactivation Fluid	1 X 402 mL
APTIMA Oil Reagent	1 X 24.6 mL

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 (1.25 mL, 5.0 mL, 12.5 mL)
2 Multi-tube vortex mixers
3 Circulating water baths (62° ± 1°C, 42° ± 1°C, 62° ± 1°C)
3 Water bath inserts
Micropipettor: 200 µL to 1000 µL
Micropipettor: 20 µL to 200 µL
Tips, Pipetman P1000 Style (Note: Special diameter tip - only available from Gen-Probe.)
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

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)
GEN-PROBE® Target Capture System (TCS) (Cat. No. 4555)
APTIMA® Auto Detect Kit (Cat. No. 1048)
APTIMA® Controls Kit (Cat. No. 301110)

STD Proficiency Panel (Cat. No. 2325)
eppendorf Repeat Pipettor (Cat. No. 2113)
Multi-tube vortex mixer (Cat. No. 2160)
Circulating water bath (Cat. No. 4586)
Water bath insert (Cat. No. 4627)
Micropipettor: 200 µL to 1000 µL (Cat. No. 4216)
Micropipettor: 20 µL to 200 µL (Cat. No. 3878)
Tips, Pipetman P1000 Style (Cat. No. 5049)
Ten Tube Units (TTU) (Cat. No. TU0022)
Ten Tip Cassettes (TTC) (Cat. No. 4578)
Replacement, non-penetrable caps (Cat. No. 3036A)
SysCheck (Cat. No. 1078)

Specimen Collection and Storage

The APTIMA GC Assay is designed to detect the presence of *N. gonorrhoeae* 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. A unisex swab is used for both male urethral and female endocervical specimens. The APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens, the APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens, and the APTIMA Vaginal Swab Specimen Collection Kit are intended to be used only with the GEN-PROBE APTIMA Combo 2 Assay, the APTIMA CT Assay, and the APTIMA GC Assay. 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° to 30°C and tested within 60 days of collection.

Urine specimens can be transported to the laboratory at 2° 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 GEN-PROBE specimen transport tube within 24 hours of collection and before being assayed. After transfer, urine specimens can be stored at 2° to 30°C for up to 30 days after collection.

Specimen collection instructions for endocervical swab, vaginal swab and 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° to 30°C until tested. Specimens must be assayed with the APTIMA GC Assay within 60 days of collection. If longer storage is needed, freeze at -20° 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° to 30°C and store at 2° to 30°C until tested. Processed urine specimens should be assayed with the APTIMA GC Assay within 30 days of collection. If longer storage is needed, freeze at -20° 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° to 30°C. Transfer the urine sample into the APTIMA urine specimen transport tube within 24 hours of collection. Store at 2° 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 the penetrable caps and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. 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° ± 1°C (for target capture, and primer annealing), a second water bath to 42° ± 1°C (for amplification), and a third water bath to 62° ± 1°C (for HPA).
   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 GC, and Probe GC 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 (Figure1, 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 (Figure 1, Step 6).
      f. Discard both the reconstitution collar and glass vial (Figure 1, 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.

h. Discard reconstituted reagent after 30 days or by the expiration date, whichever comes first.

2. If using previously reconstituted Probe GC, Amplification GC, and Enzyme Reagents, 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 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 GC and Target Capture Reagent B (TCR GC 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 GC (TCR GC) and Target Capture Reagent B (TCR-B) as follows:

Volume of TCR GC (mL)=(number of reactions+5 extra reactions)x0.1 mL

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

TCR GC plus TCR-B Preparation (Example)

Number of Reactions	TCR GC	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 GC to an appropriately sized, dedicated, clean, dry container and, using a micropipettor, add the calculated volume of TCR-B into the TCR GC.

d. Thoroughly mix the solution by swirling.

e. The TCR GC and TCR-B solution is stable for 30 days when stored at 15° to 30°C. 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 *Warnings and Precautions* 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 GC plus TCR-B reagent. Using the repeat pipettor, add 100 µL into each reaction tube.
7. Hold the Positive Control, CT/Negative Control, GC 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 Positive Control, CT/Negative Control, GC to the first reaction tube. In the same manner, add 400 µL of the Positive Control, GC/Negative Control, CT to the second reaction tube. Continue to add 400 µL of each specimen into 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 should be 400 µL ± 100 µL. See *Control and Specimen Pipetting* in *Procedural Notes* on page 7.
8. 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*.

9. Cover the TTUs with sealing cards and shake the rack gently by hand. **Do not vortex.** Incubate the rack at 62° ± 1°C in a water bath for 30 ± 5 minutes.
10. Remove the rack from the water bath and blot the bottoms of the tubes dry on absorbent material.
11. Ensure the sealing cards are firmly seated. If necessary, replace them with new sealing cards and seal the TTUs tightly.
12. Vortex the rack for 60 seconds on the multi-tube vortex mixer. See *Vortexing* in *Procedural Notes* on page 7. Begin vortexing within 2 minutes of removal of the rack from the water bath.
13. Without removing the sealing cards, incubate the rack at room temperature for 30 ± 5 minutes.
14. Place the rack on the TCS magnetic base for 5 to 10 minutes.
15. 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.

16. 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 reads greater than 25 inches Hg*. It may take 15 seconds to achieve this reading. Reconnect the manifold, and ensure the vacuum gauge is between 9.5 and 12 inches Hg*. Leave the vacuum pump on until all target capture steps are completed.

    Note: *At altitudes of 3,000 feet or higher, a Gen-Probe representative will determine the appropriate vacuum gauge reading specification.

17. 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.
18. 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.
19. 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.
20. 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.
21. Place the rack on the TCS magnetic base for 5 to 10 minutes.
22. Aspirate all liquid as in steps 17 and 18.
23. 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.

D. Amplification

1. Using the repeat pipettor, add 75 µL of the reconstituted Amplification Reagent GC 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° ± 1°C for 10 ± 5 minutes.
5. Transfer the rack into a water bath at 42° ± 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° ± 1°C for 60 ± 15 minutes.

E. Hybridization Protection Assay (HPA)

The repeat pipettor used in hybridization and selection should be dedicated for these steps only. See *Warnings and Precautions* 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 GC, 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° ± 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° ± 1°C for 10 ± 1 minutes.
    c. Remove the rack from the water bath.
3. Detection

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

    a. Incubate the rack at 18° 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 GC Assay Protocol** and enter the number of tubes (controls and specimens). Click **NEXT** to begin the run.

        Note: The run must be completed within two 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 GC Assay according to the *Test Procedure* on page 4.

Interpretation

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

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, CT/Negative Control, GC must be in the first position of the first TTU. The Positive Control, GC/Negative Control, CT 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, CT/Negative Control, GC serves as the negative control for the APTIMA GC Assay.

B. Control and Specimen Pipetting

The volume of control or specimen added to the TTU should be 400 mL ± 100 mL. 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 GC 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° 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* on page 4.

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 GC 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 at a rate capable of raising the solution into 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 the 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 the speed to the 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. Add 400 mL of bleach. 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 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 and then dry 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* on page 7.
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, CT/Negative Control, GC is positive or equivocal for GC. see *Assay Contamination* on page 7 and/or *Procedural Notes* on page 7.

Test Interpretation - QC/Patient Result

A. Test Interpretation

Assay test results are automatically interpreted by the APTIMA Assay Software using the GC protocol. A test result may be a 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	1 to < 50
Equivocal	50 to < 100
Low RLU Positive[1,2,3]	100 to < 2,000
Positive[1,2]	2,000 to < 12,000
Invalid	<1 or >12,000

[1]According to CDC guidelines, "consideration should be given to routine additional testing for persons with positive *C. trachomatis* or *N. gonorrhoeae* 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 (1).

[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

Controls must be tested with each assay run. The APTIMA Positive Control, CT/Negative Control, GC and the APTIMA 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, GC/ Negative Control, CT contains non-infectious *N. gonorrhoeae* rRNA. If desired, additional controls can be ordered as a kit. See *Materials Available from Gen-Probe* on page 3. The APTIMA Assay Controls produce the following test results:

Control	Total RLU (x1000)	GC Result
Positive Control, CT/ Negative Control, GC	≥1 and < 50	GC Negative
Positive Control, GC/ Negative Control, CT	≥100 and < 12,000	GC Positive

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 trend its values for the controls and maintain records according to standard laboratory quality control practices. Any trend variations should be investigated (13).

See *Troubleshooting* on page 7, or call Gen-Probe Technical Support for help with out-of-range controls.

C. Specimen Preparation Control (optional)

The APTIMA Positive Control, CT/Negative Control, GC and the APTIMA Positive Control, GC/Negative Control, CT 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 Result* on page 8.

D. Specimen Acceptability

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.

E. 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

GC Pos*	Positive for *N. gonorrhoeae* rRNA.
GC Neg	Presumed negative for *N. gonorrhoeae* rRNA.
GC Equiv	Sample should be retested.
Invalid	Sample should be retested.

   b. Retest results

GC Pos*	Positive for *N. gonorrhoeae* rRNA.
GC Neg	Presumed negative for *N. gonorrhoeae* rRNA.
GC 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* on this page.

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 GC test results for asymptomatic individuals or any individuals in low prevalence populations.
- A negative result does not preclude the presence of a *N. gonorrhoeae* 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 *N. gonorrhoeae*.

B. The presence of mucus in endocervical samples does not interfere with APTIMA GC Assay testing. However, to ensure proper endocervical sampling, excess mucus should be removed.

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 GC 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 (1).

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 GEN-PROBE APTIMA specimen collection kit.

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

H. Results from the APTIMA GC 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 GC 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 *N. gonorrhoeae* 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. The APTIMA GC Assay has not been validated for use with vaginal swab specimens collected by patients at home.

O. Performance of the vaginal swab specimen has not been established in pregnant women.

P. Performance of the vaginal swab specimen has not been established in teenage women less than 16 years of age.

Q. Testing of urethral swab specimens from asymptomatic males is not recommended because of the low predictive value of a positive result observed in the clinical study.

Expected Values

Prevalence

The prevalence of *N. gonorrhoeae* 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 *N. gonorrhoeae* in North America, by specimen type as determined by the APTIMA GC Assay is shown in Table 1 by clinical site and overall. Refer to the *Clinical Specimen Study* section on page 12 for a description of the clinical specimen performance characteristics.

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 GC Assay are shown in Table 2. These calculations are based on hypothetical prevalence rates and the overall sensitivity and specificity calculated from the patient infected status. The overall sensitivity and specificity for *N. gonorrhoeae* was 97.7% and 99.0%, 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 *N. gonorrhoeae* in North America by Clinical Site and Overall as Determined by APTIMA GC Assay Results

	% (#positive / #tested)											
Site	**MS**		**MU**		**FS**		**FU**		**PVS**		**CVS**	
1	21.4	(54/252)	21.4	(54/252)	6.1	(14/229)	5.7	(13/230)	6.4	(14/219)	6.1	(14/230)
2	26.5	(93/351)	20.1	(71/354)	16.1	(32/199)	15.0	(30/200)	16.2	(32/198)	16.6	(33/199)
3	0.0	(0/4)	0.0	(0/4)	4.4	(5/114)	3.5	(4/113)	3.6	(4/111)	3.5	(4/113)
4	N/A		N/A		2.3	(6/266)	1.9	(5/270)	2.2	(6/267)	3.0	(8/269)
5	5.5	(11/200)	5.5	(11/200)	1.5	(3/199)	1.0	(2/199)	1.0	(2/199)	1.0	(2/199)
6	14.5	(44/304)	13.4	(41/305)	8.2	(24/294)	5.7	(17/296)	8.3	(24/290)	7.5	(22/295)
7	5.8	(12/207)	5.8	(12/207)	0.0	(0/102)	0.0	(0/102)	0.0	(0/102)	0.0	(0/102)
8	N/A		N/A		2.0	(1/49)	2.0	(1/49)	2.1	(1/48)	2.0	(1/51)
All	16.2	(214/1318)	14.3	(189/1322)	5.9	(85/1452)	4.9	(72/1459)	5.8	(83/1434)	5.8	(84/1458)

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 in North America

Hypothetical Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
2	97.7	99.0	66.2	100
5	97.7	99.0	83.5	99.9
10	97.7	99.0	91.4	99.7
15	97.7	99.0	94.4	99.6
20	97.7	99.0	96.0	99.4
25	97.7	99.0	97.0	99.2
30	97.7	99.0	97.6	99.0

APTIMA GC Assay RLU Distribution

Figure 2 shows the RLU distribution for the APTIMA GC 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 GC Assay

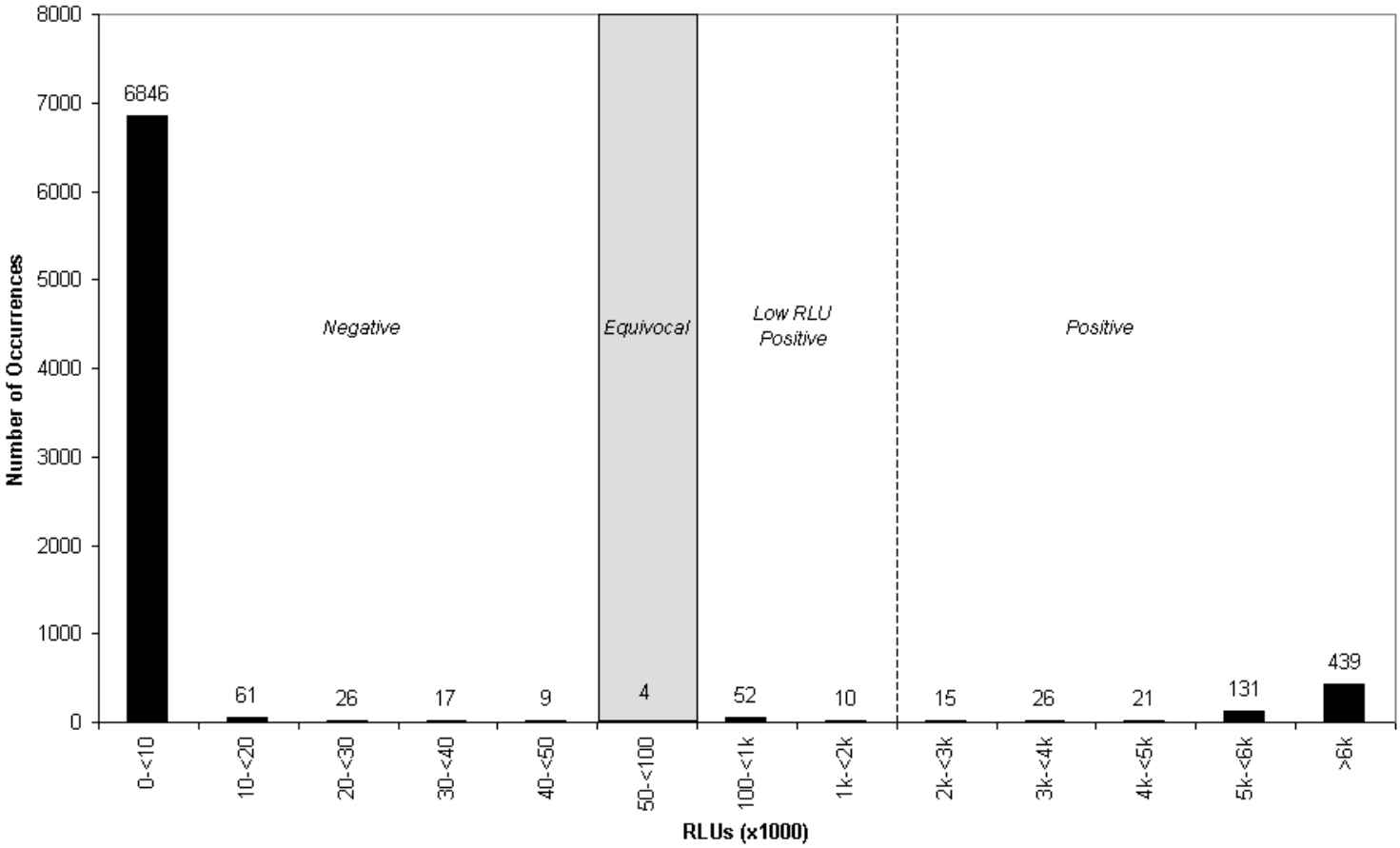

Table 3. APTIMA GC Assay RLU Distribution

	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						-	**52**	**10**	**15**	**26**	**21**	**131**	**439**
Total False Positives						-	**40**	**6**	**3**	**4**	**0**	**3**	**1**
CVS						1	5	3	0	1	0	2	0
PVS						0	7	0	1	1	0	1	1
FS						2	12	1	0	0	0	0	0
MS						1	9	0	1	0	0	0	0
FU						0	2	0	0	1	0	0	0
MU						0	5	2	1	1	0	0	0
Total Negatives	**6846**	**61**	**26**	**17**	**9**	-							
Total False Negatives	**8**	**2**	**1**	**3**	**1**	-							
CVS	2	0	0	0	0	-							
PVS	1	0	0	1	0	-							
FS	0	0	0	1	1	-							
MS	0	1	0	0	0	-							
FU	3	1	1	1	0	-							
MU	2	0	0	0	0	-							

CVS = Clinician-Collected Vaginal Swab; **PVS** = Patient-Collected Vaginal Swab; **FS** = Female Endocervical Swab; **MS** = Symptomatic 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 GC Assay and APTIMA Combo 2 Assay GC 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 GC Assay results for clinician-collected 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 *N. gonorrhoeae* was based on swab and urine specimen results from the commercially-available APTIMA Combo 2 Assay and the other commercially-available NAAT. Subjects were considered infected with *N. gonorrhoeae* if two of the four 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. Culture was not used as a reference test.

A total of 7,653 APTIMA GC Assay results were used to calculate sensitivity and specificity. Sensitivity and specificity for *N. gonorrhoeae* by gender, specimen type and symptom status, as appropriate, are presented in Table 4. Table 5 shows the APTIMA GC Assay sensitivity, specificity, and predictive values compared to patient infected status for each clinical site and overall. Tables 6a-6c summarize the number of results from symptomatic and asymptomatic subjects designated as infected or non-infected with *N. gonorrhoeae* according to the patient infected status algorithm.

Of the 2,787 subjects enrolled, there were 15 subjects with unknown GC patient infected status. Subjects were designated with an unknown patient infected status if results were missing that prevented conclusive determination of infected status. These subjects' results were not included in any performance calculations. Of the 7,704 APTIMA GC Assay results, there were 22 specimens (0.29%) that initially produced invalid or equivocal assay results. Upon retesting these specimens, 4 remained equivocal and were excluded from the analyses. The remaining 18 specimens produced valid test results upon retesting and were used in the clinical performance calculations.

Table 4. Sensitivity and Specificity of the APTIMA GC 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.)	
MS	Symptomatic	575	171	10[a]	393	1	99.4	(96.8 - 100)	97.5	(95.5 - 98.8)
MU	Symptomatic	576	171	4[b]	400	1	99.4	(96.8 - 100)	99.0	(97.5 - 99.7)
	Asymptomatic	745	9	5[c]	730	1	90.0	(55.5 - 99.7)	99.3	(98.4 - 99.8)
	All	1321	180	9[d]	1130	2	98.9	(96.1 - 99.9)	99.2	(98.5 - 99.6)
FS	Symptomatic	805	52	8[e]	744	1	98.1	(89.9 - 100)	98.9	(97.9 - 99.5)
	Asymptomatic	635	20	5[f]	609	1	95.2	(76.2 - 99.9)	99.2	(98.1 - 99.7)
	All	1440	72	13[g]	1353	2	97.3	(90.6 - 99.7)	99.0	(98.4 - 99.5)
FU	Symptomatic	810	48	2[h]	755	5	90.6	(79.3 - 96.9)	99.7	(99.0 - 100)
	Asymptomatic	639	21	1[i]	616	1	95.5	(77.2 - 99.9)	99.8	(99.1 - 100)
	All	1449	69	3[j]	1371	6	92.0	(83.4 - 97.0)	99.8	(99.4 - 100)
PVS	Asymptomatic	629	21	4[k]	604	0	100	(83.9 - 100)	99.3	(98.3 - 99.8)
	All	1422	72	11[l]	1337	2	97.3	(90.6 - 99.7)	99.2	(98.5 - 99.6)
CVS	Symptomatic	809	52	7[m]	749	1	98.1	(89.9 - 100)	99.1	(98.1 - 99.6)
	Asymptomatic	637	21	4[n]	611	1	95.5	(77.2 - 99.9)	99.3	(98.3 - 99.8)
	All	1446	73	11[o]	1360	2	97.3	(90.7 - 99.7)	99.2	(98.6 - 99.6)

N = Negative; **TP** = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.
MS = Male Urethral Swab; **MU** = Male Urine; **FS** = Female Endocervical Swab; **FU** = Female Urine;
PVS = Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab.

APTIMA Combo 2 Assay GC results: # positive results / # specimens tested a: 2/10 b: 1/4 c: 1/5 d: 2/9 e: 5/8 f: 2/5 g: 7/13 h: 1/2 i: 1/1 j: 2/3 k: 3/4 l: 8/11 m: 6/7 n: 3/4 o: 9/11.

Table 5. Sensitivity, Specificity, and Predictive Values of the APTIMA GC 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 (%)
MS	1	145	49	0	96	0	33.8	100	(92.7 - 100)	100	(96.2 - 100)	100	100
	2	177	66	8	102	1	37.9	98.5	(92.0 - 100)	92.7	(86.2 - 96.8)	89.2	99.0
	3	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
	5	49	7	1	41	0	14.3	100	(59.0 - 100)	97.6	(87.4 - 99.9)	87.5	100
	6	150	37	1	112	0	24.7	100	(90.5 - 100)	99.1	(95.2 - 100)	97.4	100
	7	54	12	0	42	0	22.2	100	(73.5 - 100)	100	(91.6 - 100)	100	100
	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
	All	575	171	10	393	1	29.9	99.4	(96.8 - 100)	97.5	(95.5 - 98.8)	94.5	99.7
MU	1	252	53	1	198	0	21.0	100	(93.3 - 100)	99.5	(97.2 - 100)	98.1	100
	2	353	68	3	280	2	19.8	97.1	(90.1 - 99.7)	98.9	(96.9 - 99.8)	95.8	99.3
	3	4	0	0	4	0	0.0	N/A		100	(39.8 - 100)	N/A	100
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
	5	200	8	3	189	0	4.0	100	(63.1 - 100)	98.4	(95.5 - 99.7)	72.7	100
	6	305	39	2	264	0	12.8	100	(91.0 - 100)	99.2	(97.3 - 99.9)	95.1	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
	All	1321	180	9	1130	2	13.8	98.9	(96.1 - 99.9)	99.2	(98.5 - 99.6)	95.2	99.8
FS	1	226	12	2	212	0	5.3	100	(73.5 - 100)	99.1	(96.7 - 99.9)	85.7	100
	2	197	29	3	164	1	15.2	96.7	(82.8 - 99.9)	98.2	(94.8 - 99.6)	90.6	99.4
	3	114	4	1	109	0	3.5	100	(39.8 - 100)	99.1	(95.0 - 100)	80.0	100
	4	260	5	1	254	0	1.9	100	(47.8 - 100)	99.6	(97.8 - 100)	83.3	100
	5	199	2	1	196	0	1.0	100	(15.8 - 100)	99.5	(97.2 - 100)	66.7	100
	6	294	19	5	269	1	6.8	95.0	(75.1 - 99.9)	98.2	(95.8 - 99.4)	79.2	99.6
	7	102	0	0	102	0	0.0	N/A		100	(96.4 - 100)	N/A	100
	8	48	1	0	47	0	2.1	100	(2.5 - 100)	100	(92.5 - 100)	100	100
	All	1440	72	13	1353	2	5.1	97.3	(90.6 - 99.7)	99.0	(98.4 - 99.5)	84.7	99.9
FU	1	227	11	2	213	1	5.3	91.7	(61.5 - 99.8)	99.1	(96.7 - 99.9)	84.6	99.5
	2	198	30	0	167	1	15.7	96.8	(83.3 - 99.9)	100	(97.8 - 100)	100	99.4
	3	113	4	0	109	0	3.5	100	(39.8 - 100)	100	(96.7 - 100)	100	100
	4	265	5	0	260	0	1.9	100	(47.8 - 100)	100	(98.6 - 100)	100	100
	5	199	2	0	197	0	1.0	100	(15.8 - 100)	100	(98.1 - 100)	100	100
	6	296	16	1	275	4	6.8	80.0	(56.3 - 94.3)	99.6	(98.0 - 100)	94.1	98.6
	7	102	0	0	102	0	0.0	N/A		100	(96.4 - 100)	N/A	100
	8	49	1	0	48	0	2.0	100	(2.5 - 100)	100	(92.6 - 100)	100	100
	All	1449	69	3	1371	6	5.2	92.0	(83.4 - 97.0)	99.8	(99.4 - 100)	95.8	99.6

Table 5 (continued) Sensitivity, Specificity, and Predictive Values of the APTIMA GC 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 (%)
PVS	1	216	11	3	202	0	5.1	100	(71.5 - 100)	98.5	(95.8 - 99.7)	78.6	100
	2	196	30	2	163	1	15.8	96.8	(83.3 - 99.9)	98.8	(95.7 - 99.9)	93.8	99.4
	3	111	4	0	107	0	3.6	100	(39.8 - 100)	100	(96.6 - 100)	100	100
	4	261	5	1	255	0	1.9	100	(47.8 - 100)	99.6	(97.8 - 100)	83.3	100
	5	199	2	0	197	0	1.0	100	(15.8 - 100)	100	(98.1 - 100)	100	100
	6	290	19	5	265	1	6.9	95.0	(75.1 - 99.9)	98.1	(95.7 - 99.4)	79.2	99.6
	7	102	0	0	102	0	0.0	N/A		100	(96.4 - 100)	N/A	100
	8	47	1	0	46	0	2.1	100	(2.5 - 100)	100	(92.3 - 100)	100	100
	All	1422	72	11	1337	2	5.2	97.3	(90.6 - 99.7)	99.2	(98.5 - 99.6)	86.7	99.9
CVS	1	227	12	2	213	0	5.3	100	(73.5 - 100)	99.1	(96.7 - 99.9)	85.7	100
	2	197	30	3	163	1	15.7	96.8	(83.3 - 99.9)	98.2	(94.8 - 99.6)	90.9	99.4
	3	113	4	0	109	0	3.5	100	(39.8 - 100)	100	(96.7 - 100)	100	100
	4	263	5	3	255	0	1.9	100	(47.8 - 100)	98.8	(96.6 - 99.8)	62.5	100
	5	199	2	0	197	0	1.0	100	(15.8 - 100)	100	(98.1 - 100)	100	100
	6	295	19	3	272	1	6.8	95.0	(75.1 - 99.9)	98.9	(96.8 - 99.8)	86.4	99.6
	7	102	0	0	102	0	0.0	N/A		100	(96.4 - 100)	N/A	100
	8	50	1	0	49	0	2.0	100	(2.5 - 100)	100	(92.7 - 100)	100	100
	All	1446	73	11	1360	2	5.2	97.3	(90.7 - 99.7)	99.2	(98.6 - 99.6)	86.9	99.9

N = Negative; **TP** = True Positive **FP** = False Positive **TN** = True Negative **FN** - False Negative.
MS = Symptomatic Male Urethral Swab; **MU** = Male Urine; **FS** = Female Endocervical Swab; **FU** = Female Urine;
PVS = Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab.

Table 6a. Symptomatic Male Urethral Swab Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

Patient Infected Status	NAAT 1 (APTIMA Combo 2 Assay)		NAAT 2		APTIMA GC Assay	Total
	MS	MU	MS	MU	MS	
Infected	+	+	+	+	+	164
Infected	+	+	+	+	-	1
Infected	+	+	+	-	+	3
Infected	+	+	=	+	+	1
Infected	+	-	+	+	+	2
Infected	+	-	+	-	+	1
Non-infected	+	-	-	-	+	2
Non-infected	+	-	-	-	-	1
Non-infected	-	+	-	-	+	1
Non-infected	-	-	+	-	-	1
Non-infected	-	-	-	+	-	2
Non-infected	-	-	-	-	+	3
Non-infected	-	-	-	-	+	2
Non-infected	-	-	-	-	-	386
Non-infected	-	-	-	-	=	1
Non-infected	-	-	-	N/A	-	1
Non-infected	-	-	-	=	-	1
Non-infected	-	-	=	-	-	1
Non-infected	=	-	-	-	+	2
Total						576

N/A = Specimen not obtained or available for testing.

The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.

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

Table 6b. Male Urine Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

Patient Infected Status	NAAT 1 (APTIMA Combo 2 Assay)		NAAT 2		APTIMA GC Assay	Symptom Status		Total
	MS	MU	MS	MU	MU	Sympt.	Asympt.	
Infected	+	+	+	+	+	164	8	172
Infected	+	+	+	+	+	1	0	1
Infected	+	+	+	-	+	3	1	4
Infected	+	+	=	+	+	1	0	1
Infected	+	-	+	+	+	2	0	2
Infected	+	-	+	-	-	1	1	2
Non-infected	+	+	-	-	+	0	1	1
Non-infected	+	-	-	-	-	2	13	15
Non-infected	+	-	-	-	-	1	0	1
Non-infected	-	+	-	-	+	1	0	1
Non-infected	-	+	-	-	-	0	1	1
Non-infected	-	-	+	-	-	1	1	2
Non-infected	-	-	-	+	-	2	2	4
Non-infected	-	-	-	-	+	3	1	4
Non-infected	-	-	-	-	-	2	1	3
Non-infected	-	-	-	-	+	0	3	3
Non-infected	-	-	-	-	-	386	691	1077
Non-infected	-	-	-	-	-	1	2	3
Non-infected	-	-	-	N/A	-	1	4	5
Non-infected	-	-	-	=	-	1	4	5
Non-infected	-	-	=	-	-	1	1	2
Non-infected	-	=	-	-	-	0	1	1
Non-infected	N/A	-	-	-	-	0	1	1
Non-infected	=	-	-	-	-	2	6	8
Non-infected	=	-	-	-	-	0	2	2
Total						576	745	1321

Sympt. = Symptomatic; **Asympt**. = Asymptomatic.

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 6c. Female Endocervical Swab and Urine Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

Patient Infected Status	NAAT 1 (APTIMA Combo 2 Assay)		NAAT 2		APTIMA GC Assay		Symptom Status		
	FS	FU	FS	FU	FS	FU	Sympt.	Asympt.	Total
Infected	+	+	+	+	+	+	43	16	59
Infected	+	+	+	+	+	-	2	0	2
Infected	+	+	+	-	+	+	2	1	3
Infected	+	+	+	-	+	-	0	1	1
Infected	+	+	+	N/A	+	+	1	0	1
Infected	+	+	-	+	+	+	1	1	2
Infected	+	+	-	-	+	+	1	1	2
Infected	+	-	+	+	+	-	1	0	1
Infected	+	-	+	-	+	+	0	1	1
Infected	+	-	+	-	+	-	2	0	2
Infected	-	+	+	+	-	+	1	0	1
Infected	-	+	-	+	-	+	0	1	1
Infected	-	+	-	+	=	+	0	1	1
Infected	-	-	+	+	-	-	1	0	1
Non-infected	+	-	-	-	+	-	4	1	5
Non-infected	+	-	-	-	-	-	1	0	1
Non-infected	-	+	-	-	-	-	1	0	1
Non-infected	-	-	+	-	+	-	1	0	1
Non-infected	-	-	+	-	-	-	5	2	7
Non-infected	-	-	-	+	-	-	2	2	4
Non-infected	-	-	-	-	+	-	1	2	3
Non-infected	-	-	-	-	-	+	1	0	1
Non-infected	-	-	-	-	-	-	718	589	1307
Non-infected	-	-	-	-	=	-	1	0	1
Non-infected	-	-	-	N/A	-	-	2	3	5
Non-infected	-	-	-	=	-	-	11	11	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	1	2
Total							811	640	1451

Sympt. = Symptomatic; **Asympt**. = Asymptomatic.

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.

Table 6d. Vaginal Swab Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

Patient Infected Status	NAAT 1 (APTIMA Combo 2 Assay)		NAAT 2		APTIMA GC Assay		Symptom Status		Total
	FS	FU	FS	FU	PVS	CVS	Sympt.	Asympt.	
Infected	+	+	+	+	+	+	43	15	58
Infected	+	+	+	+	-	+	1	0	1
Infected	+	+	+	+	-	-	1	0	1
Infected	+	+	+	+	N/A	+	0	1	1
Infected	+	+	+	-	+	+	2	2	4
Infected	+	+	+	N/A	+	+	1	0	1
Infected	+	+	-	+	+	+	1	1	2
Infected	+	+	-	-	+	+	1	1	2
Infected	+	-	+	+	+	+	1	0	1
Infected	+	-	+	-	+	+	2	1	3
Infected	-	+	+	+	+	+	1	0	1
Infected	-	+	-	+	+	+	0	1	1
Infected	-	+	-	+	+	-	0	1	1
Infected	-	-	+	+	-	-	1	0	1
Non-infected	+	-	-	-	-	-	5	1	6
Non-infected	-	+	-	-	-	-	1	0	1
Non-infected	-	-	+	-	+	+	1	0	1
Non-infected	-	-	+	-	-	-	5	2	7
Non-infected	-	-	-	+	+	+	0	1	1
Non-infected	-	-	-	+	-	-	2	1	3
Non-infected	-	-	-	-	+	+	2	1	3
Non-infected	-	-	-	-	+	-	3	1	4
Non-infected	-	-	-	-	-	+	3	1	4
Non-infected	-	-	-	-	-	-	696	577	1273
Non-infected	-	-	-	-	-	N/A	0	1	1
Non-infected	-	-	-	-	-	=	0	1	1
Non-infected	-	-	-	-	N/A	-	16	9	25
Non-infected	-	-	-	-	N/A	N/A	1	0	1
Non-infected	-	-	-	N/A	-	-	2	2	4
Non-infected	-	-	-	N/A	N/A	-	0	1	1
Non-infected	-	-	-	=	-	-	11	10	21
Non-infected	-	-	-	=	-	N/A	0	1	1
Non-infected	-	-	=	-	-	-	1	1	2
Non-infected	-	N/A	-	-	-	-	0	1	1
Non-infected	-	N/A	-	-	N/A	N/A	1	0	1
Non-infected	N/A	-	-	-	-	-	5	4	9
Non-infected	=	-	-	-	-	-	1	1	2
Total							811	640	1451

Sympt. = Symptomatic; **Asympt**. = Asymptomatic.

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; **PVS** = Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab.

RLU Distribution of APTIMA Controls

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

Table 7. RLU Distribution of APTIMA Controls

Control	Statistics	RLU (x1000)
Positive Control, GC/Negative Control, CT	Maximum	6765
	75th Percentile	5763
	Median	5175
	25th Percentile	4645
	Minimum	229
Positive Control, CT/Negative Control, GC	Maximum	20
	75th Percentile	2
	Median	2
	25th Percentile	1
	Minimum	0

Precision Study

APTIMA GC Assay precision (i.e., reproducibility) was evaluated at two external clinical sites and at Gen-Probe. APTIMA GC Assay precision was evaluated across three APTIMA GC Assay kit lots, three study sites, six operators and 108 APTIMA GC Assay runs. Two operators at each of the three testing sites performed a total of six APTIMA GC 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 2,433 fg/assay of GC rRNA. Reproducibility was established using swab transport medium spiked 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-operator, inter-lot, inter-run, and intra-run variability.

Table 8. APTIMA GC Assay Precision Data

				Inter-Site		Inter-Lot		Inter-Operator		Inter-Run		Intra-Run	
Concentration	**N**	**Mean (RLUx1000)**	**% Agrmt.**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	**CV (%)**
Neg (0 fg/mL)	540	11.7	99.8	0	N/A	0	N/A	4.3	N/A	0	N/A	233.3	N/A
Low (608-625 fg/mL)	324	5574.4	99.7	189.2	3.4	518.1	9.3	311.3	5.6	527.4	9.5	617.2	11.1
Mid (6,082 fg/mL)	108	6502.6	100	0	0.0	481.9	7.4	514.8	7.9	579.4	8.9	138.8	2.1
High (12,500 fg/mL)	324	6786.0	100	0	0.0	581.3	8.6	410.7	6.1	647.1	9.5	270.3	4.0

SD = Standard Deviation; **CV(%)** = Percent Coefficient of Variation; **% Agrmt**. = Percent Agreement.
N/A = not applicable for negative analyte.

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 (12).

Analytical Performance Characteristics

Analytical Sensitivity

N. gonorrhoeae analytical sensitivity (limit of detection) was determined by directly comparing dilutions of 51 different clinical isolates in culture and in the APTIMA GC Assay. The analytical sensitivity claim for the assay is 50 cells/assay (362 cells/swab, 250 cells/mL urine).

Analytical Specificity

A total of 154 culture isolates were evaluated using the APTIMA GC 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 papillomavirus 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 GC Assay.

Interfering Substances

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

Swab

- 10% Blood
- Contraceptive jelly
- Spermicide
- Moisturizer
- Hemorrhoidal anesthetic
- Body oil
- Powder
- Anti-fungal cream
- Vaginal lubricants
- Feminine spray
- Leukocytes (1 x 10^6 cells/mL)

Urine

- 30% Blood
- Urine analytes:
  - Protein
  - Glucose
  - Ketones
  - Bilirubin
  - Nitrate
  - Urobilinogen
- pH 4 (acidic)
- pH 9 (alkaline)
- Leukocytes (1 x 10^6 cells/mL)
- Cellular debris
- Vitamins
- Minerals
- Acetaminophen
- Aspirin
- Ibuprofen

All were tested for potential assay interference in the absence and presence of *N. gonorrhoeae* at the estimated rRNA equivalent of 50 *N. gonorrhoeae* 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 GC 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 50 *N. gonorrhoeae* cells (250 fg). These additions did not interfere with the amplification and detection of *N. gonorrhoeae* rRNA using the APTIMA GC 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 *N. gonorrhoeae* at a final concentration of approximately 50 CFU 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 *N. gonorrhoeae* 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 *N. gonorrhoeae* at a final concentration of 100 CFU per reaction. The samples were held at 30°C for 24 hours prior to being added to the urine transport media (UTM). The UTM samples then were held at 4°C and 30°C and tested in triplicate at days 1,14, 32 and 35. The UTM samples were also stored at -20°C and -70°C and tested in triplicate at days 1, 35, and 109. All replicates were positive for *N. gonorrhoeae* with UTM samples held at 4°C and -70°C. When the UTM samples were held at 30°C, 94% of the replicates were positive for *N. gonorrhoeae* at day 35. When the UTM samples were held at -20°C, 98% of the replicates were positive for *N. gonorrhoeae* at day 109.

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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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