GEN-PROBE® APTIMA COMBO 2® Assay
For *in vitro* diagnostic use

INTENDED USE

The APTIMA Combo 2 Assay is a target amplification nucleic acid probe test that utilizes target capture for the *in vitro* qualitative detection and differentiation of ribosomal RNA (rRNA) from *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* in endocervical and male urethral swab specimens, and in female and male urine specimens. The assay may be used to test specimens from symptomatic and asymptomatic individuals to aid in the diagnosis of gonococcal and/or chlamydial urogenital disease using the TIGRIS® DTS™ Automated Analyzer or semi-automated instrumentation as specified.

SUMMARY AND EXPLANATION OF THE TEST

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) infections are two of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 783,242 new cases of *C. trachomatis* and 361,705 new cases of *N. gonorrhoeae* infections were reported in the U.S. in 2001 (5).

Chlamydiae are nonmotile, gram-negative, obligate intracellular bacteria. The *C. trachomatis* 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 (22). *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and Pelvic Inflammatory Disease (PID) (3, 15, 24, 25). *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, 11, 23).

Historically, several methods for *C. trachomatis* detection have been utilized in the clinical laboratory, including cell culture, direct fluorescent antibody testing, and enzyme immunoassay. More recent methodologies for *C. trachomatis* detection include direct DNA probe assays and nucleic acid amplification (NAA) DNA probe assays. Cell culture was once considered to be the "gold standard" for detection of *C. trachomatis*. Culture is quite specific, but literature publications have demonstrated that the NAA DNA probe technologies have a higher clinical sensitivity than culture (2, 9, 17, 26). Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe and NAA assays.

N. gonorrhoeae is the causative agent of gonorrheal disease. *N. gonorrhoeae* are non-motile, gram-negative diplococci. The majority of gonorrheal infections are uncomplicated lower genital tract infections and may be asymptomatic. However, if left untreated in women, infections can ascend and cause PID. PID can manifest as endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscesses. A smaller percentage of persons with gonococcal infections may develop Disseminated Gonococcal Infection (DGI) (14, 20).

Conventional diagnosis of *N. gonorrhoeae* infection requires isolation of the organism on selective media or the observation of diplococci in Gram stained smears (16). 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 (7, 18). Commonly used non-culture methods for *N. gonorrhoeae* detection include direct DNA probe tests and NAA assays.

First generation NAA tests for *C. trachomatis* and *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, 10, 13, 19, 21, 27, 28, 29). The GEN-PROBE APTIMA Combo 2 Assay is a second generation NAA test that utilizes target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) technologies to streamline specimen processing, amplify target rRNA, and detect amplicon, respectively. Recent studies comparing performance and specimen inhibition of various amplification systems have demonstrated the benefits of target capture, TMA, and DKA technologies (8, 12). The APTIMA Combo 2 Assay qualitatively detects *C. trachomatis* and/or *N. gonorrhoeae* ribosomal ribonucleic acid (rRNA) in endocervical and male urethral swab specimens, and in female and male urine specimens from symptomatic and asymptomatic individuals.

PRINCIPLES OF THE PROCEDURE

The GEN-PROBE APTIMA Combo 2 Assay combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

Swab or urine specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the rRNA targets and protect them from degradation during storage. When the APTIMA Combo 2 Assay is performed in the laboratory, the target rRNA molecules are isolated from the urine and swab samples by the use of capture oligomers in a method called target capture; magnetic microparticles are another key feature of target capture. The capture

oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The GEN-PROBE APTIMA Combo 2 Assay replicates a specific region of the 23S rRNA from *C. trachomatis* and a specific region of the 16S rRNA from *N. gonorrhoeae* via DNA intermediates. A unique set of primers is used for each target molecule. Detection of the rRNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent DNA probes, which are complementary to a region of each target amplicon, are labeled with different acridinium ester molecules. The labeled DNA probes combine with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the *C. trachomatis* and *N. gonorrhoeae* labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for *C. trachomatis* signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for *N. gonorrhoeae* signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

REAGENTS

Reagents for the APTIMA Combo 2 Assay for *C. trachomatis* and *N. gonorrhoeae* are provided below for both the Direct Tube Sampling (DTS) System and TIGRIS DTS System. Reagent Identification Symbols are also listed next to the reagent name.

DTS System

APTIMA COMBO 2® Assay Kit (2 boxes) (Cat. No. 1032)

Refrigerated Box (2° to 8°C):

	Refrigerated Storage Tray (2° to 8°C)	
E	APTIMA Combo 2 Enzyme Reagent *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 X 100 tests
A	APTIMA Combo 2 Amplification Reagent *Nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 X 100 tests
P	APTIMA Combo 2 Probe Reagent *Non-infectious chemiluminescent DNA probes (1.2 mL/vial) dried in succinate buffered solution containing < 5% detergent.*	1 X 100 tests
TCR-B	APTIMA Combo 2 Target Capture Reagent B *Non-infectious nucleic acid in a buffered solution containing < 5% detergent.*	1 X 0.35 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
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 *cells (250 fg/assay*).*	3 X 1.7 mL

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

	Storage Tray (2° to 30°C)	
AR	APTIMA Combo 2 Amplification Reconstitution Solution *Aqueous solution containing preservatives.*	1 X 9.3 mL
ER	APTIMA Combo 2 Enzyme Reconstitution Solution *HEPES buffered solution containing a surfactant and glycerol.*	1 X 3.3 mL
PR	APTIMA Combo 2 Probe Reconstitution Solution *Succinate buffered solution containing < 5% detergent.*	1 X 12.4 mL
S	APTIMA Combo 2 Selection Reagent *600 mM borate buffered solution containing surfactant.*	1 X 31 mL

Non-Refrigerated Box (15° to 30°C):

TCR	APTIMA Combo 2 Target Capture Reagent *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

TIGRIS DTS System

APTIMA COMBO 2® Assay Kit (2 boxes) (Cat. No. 301130)

Refrigerated Box (2° to 8°C):

Refrigerated Storage Tray (2° to 8°C)

E	APTIMA Combo 2 Enzyme Reagent *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 X 4.4 mL lyophilized
A	APTIMA Combo 2 Amplification Reagent *Nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 X 10.2 mL lyophilized
P	APTIMA Combo 2 Probe Reagent *Non-infectious chemiluminescent DNA probes (3.5 mL/vial) dried in succinate buffered solution containing < 5% detergent.*	1 X 3.5 mL lyophilized
TCR-B	APTIMA Combo 2 Target Capture Reagent B *Non-infectious nucleic acid in a buffered solution containing < 5% detergent.*	1 X 0.61 mL
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*).*	5 X 1.7 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 *cells (250 fg/assay*).*	5 X 1.7 mL

Storage Tray (2° to 30°C)

AR	APTIMA Combo 2 Amplification Reconstitution Solution *Aqueous solution containing preservatives.*	1 X 27.7 mL
ER	APTIMA Combo 2 Enzyme Reconstitution Solution *HEPES buffered solution containing a surfactant and glycerol.*	1 X 11.1 mL
PR	APTIMA Combo 2 Probe Reconstitution Solution *Succinate buffered solution containing < 5% detergent.*	1 X 35.4 mL
S	APTIMA Combo 2 Selection Reagent *600 mM borate buffered solution containing surfactant.*	1 X 108 mL
	Reagent Kit Master Lot Barcode Sheet	1 sheet

Non-Refrigerated Box (15° to 30°C):

TCR	APTIMA Combo 2 Target Capture Reagent *Buffered salt solution containing solid phase (< 0.5 mg/mL) and capture oligomers.*	1 X 54 mL

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

WARNINGS AND PRECAUTIONS

A. For *in vitro* diagnostic use.

B. The assay was not evaluated in patient populations with a low prevalence of *C. trachomatis* disease, and therefore, performance in low prevalence settings has not been determined.

C. Use only supplied or specified disposable laboratory ware.

D. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. 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.

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

G. 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 and EQUIPMENT PREPARATION.

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

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

J. 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.

K. 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.

L. Adequate mixing is necessary to achieve accurate assay results. For complete details, see the PROCEDURAL NOTES section of this package insert.

M. For the collection of swab specimens, only the following collection kits have been validated for use: (i) APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens, (ii) PACE® Specimen Collection Kit for Urethral or Conjunctival Specimens (DTS System specific), and (iii) PACE Specimen Collection Kit for Endocervical Specimens (DTS System specific). Use of the PACE Collection Kit and APTIMA Adapter Kit is currently not qualified for the TIGRIS DTS System. For urine specimen collection, the APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens has been validated. Laboratories may validate other swab or urine collection devices according to the Cumitech Guide on Verification and Validation of Procedures in the Microbiology Laboratory (February, 1997, American Society for Microbiology).

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

The following warnings are DTS System specific

O. A separate area for DKA 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.

P. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a unidirectional workflow: from reagent preparation through DKA. 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 proper contamination safeguards.

Q. 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 DKA 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.

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

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

The following warning is TIGRIS DTS System specific

T. For additional specific warnings, precautions and procedures to control contamination for the TIGRIS DTS System, consult the TIGRIS DTS System Operator's Manual.

STORAGE AND HANDLING REQUIREMENTS

DTS System

A. The following reagents are stable when stored at 2° to 8°C:
APTIMA Combo 2 Enzyme Reagent
APTIMA Combo 2 Amplification Reagent
APTIMA Combo 2 Probe Reagent
APTIMA Combo 2 Target Capture Reagent B
APTIMA Positive Control CT/Negative Control GC
APTIMA Positive Control GC/Negative Control CT

B. The following reagents are stable when stored at 2° to 30°C:
APTIMA Combo 2 Amplification Reconstitution Solution
APTIMA Combo 2 Enzyme Reconstitution Solution
APTIMA Combo 2 Probe Reconstitution Solution
APTIMA Combo 2 Selection Reagent
APTIMA Wash Solution
APTIMA Buffer for Deactivation Fluid
APTIMA Oil Reagent

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

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

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

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

G. DO NOT FREEZE THE REAGENTS.

TIGRIS DTS System

A. The following reagents are stable when stored at 2° to 8°C:
APTIMA Combo 2 Enzyme Reagent
APTIMA Combo 2 Amplification Reagent
APTIMA Combo 2 Probe Reagent
APTIMA Combo 2 Target Capture Reagent B
APTIMA Positive Control, CT/ Negative Control, GC
APTIMA Positive Control, GC/ Negative Control, CT

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

APTIMA Assay Fluids must be purchased separately. APTIMA Wash Solution, APTIMA Buffer for Deactivation Fluid, APTIMA Oil Reagent, and System Fluid Preservative are available in APTIMA Assay Fluids Kit (Cat. No. 301133).

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

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

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

F. Once installed on the TIGRIS DTS System, the reagents in this kit have a 48-hour on-board stability.

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

H. DO NOT FREEZE THE REAGENTS.

MATERIALS PROVIDED

The following reagents are provided in the GEN-PROBE® APTIMA COMBO 2® Assay Kit for *C. trachomatis* and *N. gonorrhoeae* for the DTS™ System and for the TIGRIS® DTS™ System, respectively:

DTS System

APTIMA COMBO 2® Assay Kit (2 boxes) Catalog number: 1032	**100 tests**
Refrigerated box (2° to 8°C):	
Refrigerated Storage Tray	
APTIMA Combo 2 Enzyme Reagent	1 X 100 tests
APTIMA Combo 2 Amplification Reagent	1 X 100 tests
APTIMA Combo 2 Probe Reagent	1 X 100 tests
APTIMA Combo 2 Target Capture Reagent B	1 X 0.35 mL
APTIMA Positive Control CT/Negative Control GC	3 X 1.7 mL
APTIMA Positive Control GC/Negative Control CT	3 X 1.7 mL
Storage Tray (2° to 30°C)	
APTIMA Combo 2 Amplification Reconstitution Solution	1 X 9.3 mL
APTIMA Combo 2 Enzyme Reconstitution Solution	1 X 3.3 mL
APTIMA Combo 2 Probe Reconstitution Solution	1 X 12.4 mL
APTIMA Combo 2 Selection Reagent	1 X 31 mL
Reconstitution Collars	3 each
Sealing Cards	1 package

Non-Refrigerated Box (15° to 30°C):

APTIMA Combo 2 Target Capture Reagent	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

TIGRIS DTS System

APTIMA COMBO 2® Assay Kit (2 boxes) Catalog number: 301130

Refrigerated box (2° to 8°C):

Refrigerated Storage Tray

APTIMA Combo 2 Enzyme Reagent	1 X 4.4 mL lyophilized
APTIMA Combo 2 Amplification Reagent	1 X 10.2 mL lyophilized
APTIMA Combo 2 Probe Reagent	1 X 3.5 mL lyophilized
APTIMA Combo 2 Target Capture Reagent B	1 X 0.61 mL
APTIMA Positive Control, CT/ Negative Control, GC	5 X 1.7 mL
APTIMA Positive Control, GC/ Negative Control, CT	5 X 1.7 mL

Storage Tray (2° to 30°C)

APTIMA Combo 2 Amplification Reconstitution Solution	1 X 27.7 mL
APTIMA Combo 2 Enzyme Reconstitution Solution	1 X 11.1 mL
APTIMA Combo 2 Probe Reconstitution Solution	1 X 35.4 mL
APTIMA Combo 2 Selection Reagent	1 X 108 mL
Reconstitution Collars	3 each
Reagent Kit Master Lot Barcode Sheet	1 sheet

Non-Refrigerated Box (15° to 30°C):

APTIMA Combo 2 Target Capture Reagent	1 X 54 mL

MATERIALS REQUIRED BUT NOT PROVIDED

DTS System

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)
PACE® Specimen Collection Kit for Urethral or Conjunctival Specimens (Cat. No. 103275)
PACE® Specimen Collection Kit for Endocervical Specimens (Cat. No. 103300)
APTIMA® Adapter Kit (Cat. No. 301087)
GEN-PROBE® LEADER® HC+ Luminometer (Cat. No. 4747)
GEN-PROBE® Target Capture System (TCS) (Cat. No. 4555)
APTIMA® Auto Detect Kit (Cat. No. 1048)
2 Eppendorf Repeat Pipettors (Cat. No. 2113)
Repeat pipettor tips (1.25 mL, 5.0 mL, 12.5 mL)
2 Multi-tube vortex mixers (Cat. No. 2160)
3 Circulating water baths (62° ± 1°C, 42° ± 1°C, 62° ± 1°C) (Cat. No. 4586)
3 Water bath inserts (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, APTIMA Combo 2 Assay (Cat. No. 5049)
Ten Tube Units (TTU) (Cat. No. TU0022)
Ten Tip Cassettes (TTC) (Cat. No. 4578)
Tips, 1000 µL conductive, liquid sensing, TECAN 71-705 NCS
Pipette tips 20 µL to 200 µL
Clean, plastic-backed absorbent laboratory bench covers
Household bleach (sodium hypochlorite solution)
Large-capped plastic container
Standard urine collection containers, without preservatives

TIGRIS DTS System

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)
TIGRIS® DTS™ System (Cat. No. 105118)
APTIMA® Assay Fluids Kit (Cat. No. 301133)
APTIMA® Auto Detect Kit (Cat. No. 1048)
Multi-tube Units (MTU) (Cat. No. 104772)
Tips, 1000 µL conductive, liquid sensing, TECAN 71-705 NCS
Tiplet Waste Bags (Cat. No. 104215)
MTU Waste Bags (Cat. No. 104214)
MTU Waste Deflectors (Cat. No. 105258)
MTU Waste Cover (Cat. No. 105523)

Clean, plastic–backed absorbent laboratory bench covers
Household bleach (sodium hypochlorite solution)
NCCLS Type 1 water or equivalent

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)
PACE® Specimen Collection Kit for Urethral or Conjunctival Specimens (Cat. No. 103275)
PACE® Specimen Collection Kit for Endocervical Specimens (Cat. No. 103300)
APTIMA® Adapter Kit (Cat. No. 301087)
APTIMA® Controls Kit (Cat. No. 1110)
GEN-PROBE® LEADER® HC+ Luminometer (Cat. No. 4747)
GEN-PROBE® Target Capture System (TCS) (Cat. No. 4555)
APTIMA® Auto Detect Kit (Cat. No. 1048)
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)
Pipettor, Gen-Probe: 1000 µL (Rainin) (Cat. No. 4681)
Micropipettor: 200 µL to 1000 µL (Cat. No. 4216)
Micropipettor: 20 µL to 200 µL (Cat. No. 3878)
Tips, Pipetman P1000 Style, APTIMA Combo 2 Assay (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)

TIGRIS DTS System

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)
TIGRIS® DTS™ System (Cat. No. 105118)
APTIMA® Assay Fluids Kit (Cat. No. 301133)
APTIMA® Auto Detect Kit (Cat. No.1048)
STD Proficiency Panel (Cat. No. 2325)
SysCheck (Cat. No. 1078)
APTIMA® Controls Kit (Cat. No. 301110)
Multi-tube Units (MTU) (Cat. No. 104772)
Tiplet Waste Bags (Cat. No. 104215)
MTU Waste Bags (Cat. No. 104214)
MTU Waste Deflectors (Cat. No. 105258)
MTU Waste Cover (Cat. No. 105523)
Replacement, non-penetrable caps (Cat. No. 3036A)

SPECIMEN COLLECTION AND STORAGE

The APTIMA Combo 2 Assay is designed to detect the presence of *C. trachomatis* and *N. gonorrhoeae* in endocervical and male urethral specimens, and in female and male urine specimens. Only the swabs and the specimen transport tubes contained in the 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 and female specimens. The APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens. APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens, PACE Specimen Collection Kit for Urethral or Conjunctival Specimens, PACE Specimen Collection Kit for Endocervical Specimens, and the APTIMA Adapter Kit are intended to be used only with GEN-PROBE APTIMA Combo 2 Assay. Use of the PACE collection kits and APTIMA Adapter Kit is currently not qualified for the TIGRIS DTS System. 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 stability for these conditions was established with pooled samples spiked with 10 IFU CT and 100 CFU GC/assay. Samples at lower levels with both organisms, or with either organism alone were not tested in the specimen stability study.

A. Instructions for collection:

1. Endocervical swab specimens
   a. Remove excess mucus from the cervical os and surrounding mucosa using the cleaning swab (white shaft swab in the package with red printing). **Discard this swab.**

      Note: To remove excess mucus from the cervical os, a large-tipped cleaning swab (not provided) may be used. Discard swab after use.
   b. Insert the specimen collection swab (blue shaft swab in the package with green printing) into the endocervical canal.
   c. Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
   d. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
   e. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
   f. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
   g. Recap the swab specimen transport tube tightly.
2. Male urethral swab specimens
   a. The patient should not have urinated for at least one hour prior to specimen collection.
   b. Insert the specimen collection swab (blue shaft swab in the package with the green printing) 2 to 4 cm into the urethra.
   c. Gently rotate the swab clockwise for 2 to 3 seconds in the urethra to ensure adequate sampling.
   d. Withdraw the swab carefully.
   e. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the specimen transport tube.
   f. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
   g. Recap the swab specimen transport tube tightly.
3. Urine Specimens
   a. The patient should not have urinated for at least one hour prior to specimen collection.
   b. Direct patient to provide a first-catch urine (approximately 20 to 30 mL of the initial urine stream) into a urine collection cup free of any preservatives. Collection of larger volumes of urine may result in specimen dilution that may reduce test sensitivity. Female patients should not cleanse the labial area prior to providing the specimen.
   c. Remove the cap and transfer 2 mL of urine into the urine specimen transport tube using the disposable pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine transport tube label.
   d. Recap the urine specimen transport tube tightly. This is now known as the *processed urine specimen*.

B. Specimen transport and storage before testing:

1. Swab specimens:

   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 Combo 2 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 GEN-PROBE 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 Combo 2 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.

C. Specimen storage after testing:

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen transport tubes should be covered with a new, clean plastic or foil barrier.
3. If assayed samples need to be frozen or shipped, remove penetrable cap and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 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 in Atlanta, Georgia at 1-800-311-3435.

TEST PROCEDURE

DTS System

A. EQUIPMENT PREPARATION

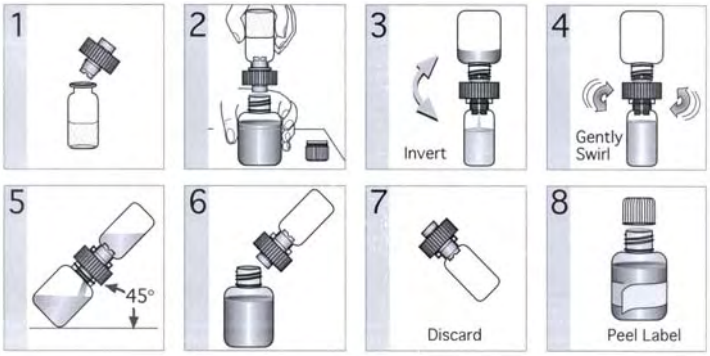
1. Adjust one water bath to 62° ± 1°C (for target capture, and primer annealing), a second water bath to 42° ± 1°C (for amplification), and a third water bath to 62° ± 1°C (for DKA).

2. Prior to starting the assay, wipe down work surfaces and pipettors with household bleach diluted 1:1 with water (1 part bleach, 1 part water). Allow bleach to contact surfaces and pipettors for at least 1 minute and 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 for use with the APTIMA Assay Operator's Manual*.)

B. REAGENT RECONSTITUTION/PREPARATION

This step should be performed prior to beginning specimen transfer.

1. To reconstitute the APTIMA Combo 2 Enzyme, Amplification, and Probe Reagents:
   a. Pair the appropriate reconstitution solution with the dried reagent. The labels have been color coded so the paired reagents have the same color bands.
   b. Open the dried reagent and firmly insert the notched end of the reconstitution collar into the glass vial (figure 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 2).
   d. Invert the assembly, allow the solution to drain into the glass container (figure 3), and then swirl gently (figure 4). Invert the assembly and tilt at a 45° angle (figure 5). Allow all of the liquid to drain back into the plastic bottle.
   e. Remove the reconstitution collar and the glass vial (figure 6).
   f. Discard both the reconstitution collar and glass vial (figure 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 8).
   h. Discard reconstituted reagent after 30 days or by the expiration date, whichever comes first.



2. If using previously reconstituted Probe, Amplification, 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. Repeat if necessary.
3. To prepare the Target Capture Reagent plus Target Capture Reagent B (TCR plus TCR-B):
   a. Determine the number of reactions to be performed (specimens plus controls).
   b. Calculate the volumes of Target Capture Reagent (TCR) and Target Capture Reagent B (TCR-B) as follows:

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

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

TCR plus TCR-B Preparation (Example)

Number of Reactions	TCR	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 to an appropriately sized, dedicated, clean, dry container and, using a micropipettor, add the calculated volume of TCR-B into the TCR.
   d. Thoroughly mix the solution by swirling.
   e. The TCR plus TCR-B 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.)

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. If the urine specimens contain precipitates, heat the specimens at 37°C for up to 5 minutes. In the event that the precipitate does not go back into solution, ensure that the precipitate does not prevent delivery of the specimen.
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 *LEADER HC+ Luminometer for APTIMA Assay Operator's Manual*.
6. Thoroughly mix the TCR 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 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 the PROCEDURAL NOTES section.
8. If specimens with standard (impenetrable) 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 for use with the APTIMA Assay 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 bottoms of tubes dry on absorbent material.
11. Ensure the sealing cards are firmly seated. If necessary, replace with new sealing cards and seal tightly.
12. Vortex rack for 60 seconds on the multi-tube vortex mixer. See "VORTEXING" in the PROCEDURAL NOTES section. Begin vortexing within 2 minutes of removal of rack from water bath.
13. Without removing 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 10 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 in. Hg*. It may take 15 seconds to achieve this reading. Reconnect the manifold, and ensure the vacuum gauge is between 9.5 and 12 in. 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 specifications.
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 tubes with a sealing card and remove the rack from the TCS. Vortex once on the multi-tube vortex mixer. See PROCEDURAL NOTES.
21. Place 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 to each reaction tube. All reaction mixtures in the rack should now be red in color.
2. Using the repeat pipettor, add 200 µL of Oil Reagent.
3. Cover tubes with a sealing card and vortex 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 in color.
7. Immediately cover the tubes with a fresh sealing card, remove 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. DUAL KINETIC ASSAY (DKA)

The repeat pipettor used in hybridization and selection should be dedicated for these steps only. (See WARNINGS AND PRECAUTIONS.)

1. HYBRIDIZATION
   a. Remove the rack from the water bath and transfer to the DKA area. Add 100 µL of the reconstituted Probe Reagent, using the repeat pipettor. All reaction mixtures should now be yellow in color.
   b. Cover tubes with a sealing card and vortex 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 at room temperature for 5 ± 1 minute.
2. SELECTION
   a. Using the repeat pipettor, add 250 µL of Selection Reagent to each tube. All reactions should now be red in color.
   b. Cover tubes with a sealing card, vortex for 10 seconds or until the color is uniform, and incubate the rack in a water bath at 62° ± 1°C for 10 ± 1 minute.
   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 Combo 2 Assay Software refer to the *LEADER HC+ Luminometer for APTIMA Assay Operator's Manual.*
   c. Prepare the LEADER HC+ Luminometer by placing one empty TTU in cassette position number 1 and perform 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** 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 time.
   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 4 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 FOR TIGRIS DTS AND DTS SYSTEMS

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

To monitor for laboratory contamination, the following procedure may be performed using the APTIMA Unisex Swab Specimen Collection Kit for 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 transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat steps 2 to 5 for all areas to be swabbed.
7. Test the swab using the APTIMA Combo 2 Assay according to the Test Procedure section of the package insert.

Interpretation:
If the results are CT or GC positive or equivocal (see TEST INTERPRETATION), the surface may be contaminated and should be decontaminated by treating with bleach as recommended in TEST PROCEDURE, EQUIPMENT PREPARATION.

Note: If contamination of the water bath is suspected, the water bath water can be tested using the procedure described for a urine specimen, by adding 2.0 mL of the water to a urine specimen transport tube (DTS System specific).

See TIGRIS DTS System Operator's Manual for additional TIGRIS DTS System-specific contamination monitoring information.

Test Procedure for TIGRIS DTS System

For equipment preparation, reagent reconstitution/preparation and rack setup information, see TIGRIS DTS System Operator's Manual sections STORING AND PREPARING ASSAY REAGENTS (ARs) and OPERATING THE TIGRIS DTS SYSTEM.

PROCEDURAL NOTES

DTS System

A. CONTROLS

To work properly with the APTIMA Combo 2 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.

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, repipette the Target Capture Reagent and the control or specimen into a new tube.

C. REAGENTS

Probe Reconstitution Solution may precipitate upon storage. Warming and mixing the solution at 62° ± 1°C will dissolve the precipitate.

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.

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 Combo 2 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 lifting 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. 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.
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 Positive Control, CT/Negative Control, GC is positive or equivocal for GC or the Positive Control, GC/Negative Control, CT is positive or equivocal for CT, see ASSAY CONTAMINATION.

TIGRIS DTS System

See TIGRIS DTS System Operator's Manual for additional TIGRIS DTS System procedural information.

TEST INTERPRETATION - QC/PATIENT RESULT

TIGRIS DTS and DTS Systems

A. TEST INTERPRETATION

Assay test results are automatically interpreted by the APTIMA Combo 2 Assay Software and presented as individual CT and GC test results. A test result may be a negative, equivocal, positive, or invalid as determined by the kinetic type and total RLU in the detection step (see following table). A test result may be invalid due to a parameter outside the normal expected ranges. Initial equivocal and invalid test results should be repeated.

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

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

B. QUALITY CONTROL RESULTS AND ACCEPTABILITY

Controls must be run with each assay. 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, CT/Negative Control GC serves as the negative control for the GC test results. The Positive Control, GC/Negative Control, CT serves as the negative control for the CT test results. If desired, a dual negative control furnished by the user can be added to monitor assay background. Correct preparation of specimens is confirmed visually by the presence of a single 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 Positive Controls must produce the following test results:

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

1. The APTIMA Combo 2 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 (DTS System specific).
2. If the Run Status is FAIL, all test results in the same run are invalid and must not be reported (DTS System specific).
3. Each laboratory should trend its values for the Positive Control CT/Negative Control GC and Positive Control GC/Negative Control CT and maintain records according to standard laboratory quality control practices. Any trend variations should be investigated. Refer to NCCLS EP12-A: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline for additional guidance on appropriate internal quality control testing practices (National Committee for Clinical Laboratory Standards. NCCLS, Wayne, PA.).
4. A TIGRIS DTS System parameter permits each site to specify a "control bracketing" frequency whereby additional sets of controls can be placed at defined intervals within the worklist. If this parameter is specified, the TIGRIS DTS System will require a set of controls to be placed after the defined number of specimens in the control bracket. The TIGRIS DTS System automatically evaluates each control in the worklist according to the above criteria and will invalidate all specimens in the affected control bracket(s) if the control criteria are not met. See the TIGRIS System Operator's Manual for additional details.
5. Negative controls may not be effective in monitoring random carryover. See TIGRIS DTS SYSTEM ANALYTICAL PERFORMANCE Section for results from a high-target analytical carryover study that was performed to demonstrate control of carryover on the TIGRIS DTS System.

See TROUBLESHOOTING section 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 provided in the kit act as controls for the TARGET CAPTURE, AMPLIFICATION, and DETECTION steps of the assay and must be included in each assay run. If desired, controls for cell lysis and RNA stabilization 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; A. TEST INTERPRETATION).

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 *C. trachomatis* rRNA
CT Neg	presumed negative for *C. trachomatis* rRNA
CT Equiv	sample should be retested
GC Pos	positive for *N. gonorrhoeae* rRNA
GC Neg	presumed negative for *N. gonorrhoeae rRNA*
GC Equiv	sample should be retested

   b. Retest results

CT Pos	positive for *C. trachomatis* rRNA
CT Neg	presumed negative for *C. trachomatis* rRNA
CT Equiv	indeterminate, a new specimen should be collected
GC Pos	positive for *N. gonorrhoeae* rRNA
GC Neg	presumed negative for *N. gonorrhoeae* rRNA
GC Equiv	indeterminate, a new specimen should be collected

NOTES:

- Careful consideration of performance data is recommended for interpreting APTIMA Combo 2 Assay results for asymptomatic individuals or any individuals in low prevalence populations.
- The first valid result for each analyte is the result that should be reported.
- A negative result does not preclude the presence of a *C. trachomatis* or *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, or specimen mix-up.

- As is true for all non-culture methods, a positive specimen obtained from a patient after therapeutic treatment cannot be interpreted as indicating the presence of viable *C. trachomatis* or *N. gonorrhoeae*.

- As is true for all urine test methods, a negative urine result for a female patient who is clinically suspected of having a chlamydial or gonococcal infection does not rule out the presence of *C. trachomatis* or *N. gonorrhoeae* in the urogenital tract. Testing of an endocervical specimen is recommended in such cases. As well, a negative urine result for *N. gonorrhoeae* from a female has a lower negative predictive value than does an endocervical swab result.

LIMITATIONS

A. Swab specimens were evaluated in the APTIMA Combo 2 Assay on the DTS System for interference by blood, gynecological lubricants, and spermicides. Urine specimens were evaluated for interference by blood, commonly used vitamins, minerals, and over-the-counter pain relievers. Blood interference also was evaluated on the TIGRIS DTS System. The data indicated no assay interference by these substances.

B. The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of *C. trachomatis* or *N. gonorrhoeae*.

C. The presence of mucus in endocervical samples does not interfere with the detection of *C. trachomatis* or *N. gonorrhoeae* by the APTIMA Combo 2 Assay. However, to ensure collection of cells infected with *C. trachomatis*, columnar epithelial cells lining the endocervix should be sampled. If excess mucus is not removed, sampling of these cells is not ensured.

D. 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.

E. This method has been tested using endocervical and male urethral swab specimens and female and male urine specimens only. Performance with other specimens has not been assessed. Specimens other than those collected with the APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens, the APTIMA Urine Collection Kit for Male and Female Urine Specimens, PACE Specimen Collection Kit for Urethral or Conjunctival Specimens, PACE Specimen Collection Kit for Endocervical Specimens, and the APTIMA Adapter Kit have not been evaluated.

F. Urine 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.

G. The APTIMA Combo 2 Assay is not intended for the evaluation of suspected child sexual abuse. For those patients for whom a false positive result may have adverse psycho-social impact, or if the positive predictive value of the test is less than 90%, CDC recommends retesting by a method using an alternate nucleic acid amplification technology or the same technology but targeting an alternate nucleic acid sequence (4).

H. Reliable results are dependent on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. See the SPECIMEN COLLECTION AND STORAGE section of this insert for instructions.

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

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

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

L. The APTIMA Combo 2 Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the number of organisms in a specimen.

M. Performance characteristics for detecting *C. trachomatis* and *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.

N. The performance of the TIGRIS DTS System with the APTIMA Combo 2 Assay has not been determined at altitudes above 2700 feet. Additional volumetric verifications and assay specific studies will be performed prior to, or as part of, the installation and acceptance process in laboratories above 2700 ft altitude. (TIGRIS DTS System specific)

O. Customers must independently validate an LIS transfer process (TIGRIS DTS System specific).

EXPECTED VALUES

DTS System

Prevalence

The prevalence of *C. trachomatis* and/or *N. gonorrhoeae* disease in patient populations depends on risk factors such as age, gender, the presence of symptoms, the type of clinic, and the test method. A summary of the prevalence of three *C. trachomatis* and *N. gonorrhoeae* disease outcomes as determined by the APTIMA Combo 2 Assay is shown in Table 1.

Table 1:
Prevalence of *C. trachomatis* and/or *N. gonorrhoeae* Disease as Determined by the APTIMA Combo 2 Assay Results by Clinical Site

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

Positive and Negative Predictive Values

The hypothetical positive and negative predictive values (PPV and NPV) for different prevalence rates using the APTIMA Combo 2 Assay are shown in Tables 2 and 3. These calculations are based on a hypothetical prevalence and the overall sensitivity and specificity calculated from the patient infected status. The overall sensitivity and specificity for *C. trachomatis* was 95.8% and 98.2%, respectively (Table 2). The overall sensitivity and specificity for *N. gonorrhoeae* was 97.8% and 98.9%, respectively (Table 3). The actual PPV and NPV calculated using the clinical trial data are shown in Tables 5 and 9.

Table 2: Hypothetical PPV and NPV for *C. trachomatis*

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
2	95.9	98.2	52.1	99.9
5	95.9	98.2	73.7	99.8
10	95.9	98.2	85.5	99.5
15	95.9	98.2	90.4	99.3
20	95.9	98.2	93.0	99.0
25	95.9	98.2	94.7	98.6
30	95.9	98.2	95.8	98.2

Table 3: Hypothetical PPV and NPV for *N. gonorrhoeae*

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
2	97.8	98.9	64.5	100
5	97.8	98.9	82.4	99.9
10	97.8	98.9	90.8	99.8
15	97.8	98.9	94.0	99.6
20	97.8	98.9	95.7	99.4
25	97.8	98.9	96.7	99.3
30	97.8	98.9	97.4	99.1

DTS SYSTEM PERFORMANCE CHARACTERISTICS

Clinical Trial Results

Performance characteristics for the APTIMA Combo 2 Assay were established in a multi-center study at seven geographically diverse clinical sites. The study evaluated swab and urine specimens from 1,363 male subjects and 1,569 female subjects attending STD, OB/GYN and Family Planning Clinics. A total of 15,661 *C. trachomatis* and 14,144 *N. gonorrhoeae* test results were used in the data analysis. As many as three urethral swabs and a urine specimen were collected from male subjects and four endocervical swabs and a urine specimen were collected from female subjects. For males providing one urethral swab, testing included *N. gonorrhoeae* culture only. For males providing three swabs, testing included *N. gonorrhoeae* culture, the APTIMA Combo 2 Assay, and a commercially available amplified nucleic acid assay for *C. trachomatis* and *N. gonorrhoeae*. Testing on endocervical swabs included the APTIMA Combo 2 Assay, two commercially available amplified *C. trachomatis* assays, one commercially available amplified *N. gonorrhoeae* assay, and *N. gonorrhoeae* culture. The *N. gonorrhoeae* culture swab was collected first and the collection order for the remaining swabs was rotated among the remaining assays to minimize collection bias. Urine was tested by the APTIMA Combo 2 Assay, two commercially available amplified assays for *C. trachomatis*, and one commercially available amplified assay for *N. gonorrhoeae*. The commercially available amplification assays were used as comparator assays in the APTIMA Combo 2 clinical trial.

All performance calculations were based on the total number of APTIMA Combo 2 Assay swab and urine specimens compared to patient infected status. The designation of a subject being infected, not infected, or inconclusive was based on the combined results of the comparator assay swab and urine results. For *C. trachomatis* infected status, any two positive comparator assay results by any combination of swab and urine designated the subject as infected. If all comparator results were negative, the subject was designated not infected. If there was one positive result only, the subject was designated inconclusive. For *N. gonorrhoeae* infected status, a positive culture, or positive swab and urine results by the amplified comparator assay, designated the subject as infected. A negative culture and a single positive result by the amplified comparator assay resulted in an inconclusive status. If all comparator results were negative, the subject was designated not infected. Tables 6, 7, 10 and 11 summarize the frequency of test outcomes for the two comparator assays and APTIMA Combo 2 Assay for clinical trial subjects.

C. trachomatis performance estimates by gender, specimen type and symptomatic status are presented in Table 4. Table 5 shows the *C. trachomatis* performance estimates for the APTIMA Combo 2 Assay compared to patient infected status for each clinical site. Performance estimates for detection of *N. gonorrhoeae* by gender, specimen type and symptomatic status are presented in Table 8. Table 9 shows the *N. gonorrhoeae* performance estimates for the APTIMA Combo 2 Assay compared to patient infected status for each clinical site. Samples that were APTIMA Combo 2 Assay positive, infected patient status negative (i.e., apparent false positives) were tested in alternate amplification assays for *C. trachomatis* and *N. gonorrhoeae*. These assays are GEN-PROBE research assays that amplify *C. trachomatis* and *N. gonorrhoeae* sequences unique from those amplified in the APTIMA Combo 2 Assay. Testing was done on a per specimen basis (i.e., not necessarily on paired swab and urine specimens) and the results of the alternate amplification assays were not used to change the original patient categorizations (Tables 4 and 8).

Specimens with missing comparator assay results, single positive results, or insufficient numbers of specimens were originally classified as inconclusive during the clinical trial. As part of a subsequent data analysis, these inconclusive specimens were reclassified as non-infected. Percent agreement of the APTIMA Combo 2 Assay results with patient infected status for those patients having both *C. trachomatis* and *N. gonorrhoeae* results was calculated (Table 12). Using these criteria, 1,186 males with APTIMA Combo 2 Assay swab tests and 1,318 males with APTIMA Combo 2 Assay urine tests could be evaluated. Similarly, 1,548 females with APTIMA Combo 2 Assay swab tests and 1,555 females with APTIMA Combo 2 urine tests could be evaluated.

Swab specimens were evaluated for the impact of blood on *C. trachomatis* and *N. gonorrhoeae* assay performance. Of the 2,454 specimens evaluated for *C. trachomatis* performance, 234 (9.5%) were bloody. Of the 2,829 specimens evaluated for *N. gonorrhoeae* performance, 247 (8.7%) were bloody. Neither the *C. trachomatis* or *N. gonorrhoeae* assay performance was statistically different for bloody specimens as compared to non-bloody specimens. Additional data on blood testing can be found in PERFORMANCE CHARACTERISTICS - Interfering Substances.

Performance of the assay with specimens from pregnant females was assessed in the clinical trial. For *C. trachomatis*, sensitivity for swab and urine specimens was 100% (8/8) and 100% (8/8), respectively. Specificity for swab and urine specimens was 95.8% (23/24) and 100% (24/24), respectively. For *N. gonorrhoeae*, sensitivity for swab and urine specimens was 100% (8/8) and 100% (8/8), respectively. Specificity for swab and urine specimens was 100% (26/26) and 100% (26/26), respectively.

Of the 11,406 APTIMA Combo 2 Assay test results from the multi-center clinical trial, 3 *C. trachomatis* results and 9 *N. gonorrhoeae* results were equivocal on repeat testing and were excluded from the analysis. One specimen was invalid for both *C. trachomatis* and *N. gonorrhoeae* results and was excluded from the study.

Table 4: *C. trachomatis* Sensitivity and Specificity: APTIMA Combo 2 Assay Specimens vs. Patient Infected Status

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

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

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

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

[4] CT TMA Alternate Amplification results: # positive results/# specimens tested
a: 11/14; b: 3/5; c: 4/8; d: 0/4; e: 18/22; f: 4/6; g: 2/8; h: 1/5; i: 29/36; j: 7/11; k: 6/16; l: 1/9

Table 5: *C. trachomatis* Performance by Clinical Site: APTIMA Combo 2 Assay Specimens vs. Patient Infected Status

Gender	Specimen	Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)		Specificity (95% C.I.)		PPV (%)	NPV (%)
Male	Swab	1	157	35	6	115	1	22.9	97.2%	(85.5 - 99.9)	95.0%	(89.5 - 98.2)	85.4	99.1
		2	93	19	2	72	0	20.4	100%	(82.4 - 100)	97.3%	(90.6 - 99.7)	90.5	100
		3	248	76	5	165	2	31.5	97.4%	(91.0 - 99.7)	97.1%	(93.3 - 99.0)	93.8	98.8
		4	51	12	1	38	0	23.5	100%	(73.5 - 100)	97.4%	(86.5 - 99.9)	92.3	100
		5	138	24	0	113	1	18.1	96.0%	(79.6 - 99.9)	100%	(96.8 - 100)	100	99.1
		6	353	74	6	268	5	22.4	93.7%	(85.8 - 97.9)	97.8%	(95.3 - 99.2)	92.5	98.2
		7	25	20	0	3	2	88.0*	90.9%	(70.8 - 98.9)	100%	(29.2 - 100)	100	60.0
		ALL	1065	260	20	774	11	25.4	95.9%	(92.9 - 98.0)	97.5%	(96.1 - 98.5)	92.9	98.6
	Urine	1	157	35	6	115	1	22.9	97.2%	(85.5 - 99.9)	95.0%	(89.5 - 98.2)	85.4	99.1
		2	96	22	1	73	0	22.9	100%	(84.6 - 100)	98.6%	(92.7 - 100)	95.7	100
		3	249	78	2	169	0	31.3	100%	(95.4 - 100)	98.8%	(95.8 - 99.9)	97.5	100
		4	51	12	0	39	0	23.5	100%	(73.5 - 100)	100%	(91.0 - 100)	100	100
		5	162	31	2	129	0	19.1	100%	(88.8 - 100)	98.5%	(94.6 - 99.8)	93.9	100
		6	353	74	1	273	5	22.4	93.7%	(85.8 - 97.9)	99.6%	(98.0 - 100)	98.7	98.2
		7	27	24	0	3	0	88.9*	100%	(85.8 - 100)	100%	(29.2 - 100)	100	100
		ALL	1095	276	12	801	6	25.8	97.9%	(95.4 - 99.2)	98.5%	(97.4 - 99.2)	95.8	99.3
Female	Swab	1	150	34	4	110	2	24.0	94.4%	(81.3 - 99.3)	96.5%	(91.3 - 99.0)	89.5	98.2
		2	81	11	1	68	1	14.8	91.7%	(61.5 - 99.8)	98.6%	(92.2 - 100)	91.7	98.6
		3	184	51	13	114	6	31.0	89.5%	(78.5 - 96.0)	89.8%	(83.1 - 94.4)	79.7	95.0
		4	196	27	2	167	0	13.8	100%	(87.2 - 100)	98.8%	(95.8 - 99.9)	93.1	100
		5	370	27	1	341	1	7.6	96.4%	(81.7 - 99.9)	99.7%	(98.4 - 100)	96.4	99.7
		6	274	35	7	230	2	13.5	94.6%	(81.8 - 99.3)	97.0%	(94.0 - 98.8)	83.3	99.1
		7	134	10	0	124	0	7.5	100%	(69.2 - 100)	100%	(97.1 - 100)	100	100
		ALL	1389	195	28	1154	12	14.9	94.2%	(90.1 - 97.0)	97.6%	(96.6 - 98.4)	87.4	99.0
	Urine	1	150	34	4	110	2	24.0	94.4%	(81.3 - 99.3)	96.5%	(91.3 - 99.0)	89.5	98.2
		2	81	12	1	68	0	14.8	100%	(73.5 - 100.0)	98.6%	(92.2 - 100)	92.3	100
		3	185	54	3	125	3	30.8	94.7%	(85.4 - 98.9)	97.7%	(93.3 - 99.5)	94.7	97.7
		4	196	24	2	167	3	13.8	88.9%	(70.8 - 97.6)	98.8%	(95.8 - 99.9)	92.3	98.2
		5	369	28	2	338	1	7.9	96.6%	(82.2 - 99.9)	99.4%	(97.9 - 99.9)	93.3	99.7
		6	276	35	1	238	2	13.4	94.6%	(81.8 - 99.3)	99.6%	(97.7 - 100)	97.2	99.2
		7	134	10	0	124	0	7.5	100%	(69.2 - 100)	100%	(97.1 - 100)	100	100
		ALL	1391	197	13	1170	11	15.0	94.7%	(90.7 - 97.3)	98.9%	(98.1 - 99.4)	93.8	99.1

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

Table 6: *C. trachomatis* Specimen Analysis for Female Patient Infected Status

Infected Status	Amp 1 Urine	Amp 1 Swab	Amp 2 Urine	Amp 2 Swab	Combo 2 Urine	Combo 2 Swab	Symp	Asymp
Infected	N/A	N/A	+	+	+	+	1	0
Infected	N/A	+	N/A	+	+	+	1	0
Infected	N/A	+	+	+	-	+	0	1
Infected	-	+	N/A	+	-	+	1	0
Infected	-	+	-	+	-	+	4	0
Infected	-	+	-	+	+	+	6	1
Infected	-	+	+	+	-	+	1	0
Infected	-	+	+	+	+	+	7	3
Infected	+	N/A	+	+	+	+	1	0
Infected	+	-	N/A	+	+	-	1	0
Infected	+	-	+	-	-	-	1	0
Infected	+	-	+	-	+	-	7	1
Infected	+	-	+	-	+	+	2	1
Infected	+	-	+	+	+	-	1	0
Infected	+	-	+	+	+	+	3	3
Infected	+	+	N/A	+	+	+	6	2
Infected	+	+	-	N/A	+	+	1	0
Infected	+	+	-	+	+	+	7	3
Infected	+	+	+	N/A	+	+	1	0
Infected	+	+	+	-	+	+	2	2
Infected	+	+	+	+	-	-	1	0
Infected	+	+	+	+	-	+	1	1
Infected	+	+	+	+	+	N/A	1	0
Infected	+	+	+	+	+	+	88	44
Non-infected	-	-	-	-	N/A	-	1	1
Non-infected	-	-	-	-	-	N/A	2	1
Non-infected	-	-	-	-	-	-	648	497
Non-infected	-	-	-	-	-	+	18	4
Non-infected	-	-	-	-	+	-	4	3
Non-infected	-	-	-	-	+	+	4	2
Total							822	570

N/A = Specimen not obtained or available for testing
Symp = Symptomatic
Asymp = Asymptomatic

Table 7: C. trachomatis Specimen Analysis for Male Patient Infected Status

Infected Status	Amp 1 Urine	Amp 1 Swab	Amp 2 Urine	Combo 2 Urine	Combo 2 Swab	Symp	Asymp
Infected	N/A	+	+	+	+	2	0
Infected	-	+	+	+	+	10	4
Infected	+	N/A	+	+	N/A	4	6
Infected	+	N/A	+	+	-	2	0
Infected	+	N/A	+	+	+	21	1
Infected	+	-	+	+	-	3	3
Infected	+	-	+	+	+	4	3
Infected	+	+	N/A	-	+	1	0
Infected	+	+	N/A	+	+	8	2
Infected	+	+	-	+	+	12	4
Infected	+	+	+	-	-	1	0
Infected	+	+	+	-	+	1	3
Infected	+	+	+	+	N/A	1	0
Infected	+	+	+	+	-	1	1
Infected	+	+	+	+	+	131	53
Non-infected	-	-	-	N/A	-	0	2
Non-infected	-	-	-	-	N/A	13	8
Non-infected	-	-	-	-	-	461	303
Non-infected	-	-	-	-	+	10	5
Non-infected	-	-	-	+	-	3	4
Non-infected	-	-	-	+	+	5	0
Total						694	402

N/A = Specimen not obtained or available for testing
Symp = Symptomatic
Asymp = Asymptomatic

Table 8: *N. gonorrhoeae* Sensitivity and Specificity: APTIMA Combo 2 Assay Specimens vs. Patient Infected Status

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

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

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

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

[4] GC TMA Alternate Amplification results: # positive results/# specimens tested
a: 5/5; b: 12/12; c: 0/1; d: 2/2; e: 13/15; f: 2/2; g: 4/7; h: 0/2; i: 18/20; j: 14/14; k: 4/8; l: 2/4

Table 9: *N. gonorrhoeae* Performance by Clinical Site: APTIMA Combo 2 Assay Specimens vs. Patient Infected Status

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

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

Table 10: *N. gonorrhoeae* Specimen Analysis for Female Patient Infected Status

Infected Status	Amp 1 Urine	Amp 1 Swab	Culture Swab	Combo 2 Urine	Combo 2 Swab	Symp	Asymp
Infected	N/A	+	+	+	+	1	1
Infected	-	-	+	-	-	0	1
Infected	-	+	+	-	+	5	2
Infected	-	+	+	+	+	9	2
Infected	+	N/A	+	+	+	1	0
Infected	+	-	+	+	+	3	1
Infected	+	+	N/A	+	+	0	1
Infected	+	+	-	+	+	11	2
Infected	+	+	+	-	+	2	1
Infected	+	+	+	+	+	62	21
Non-infected	-	-	-	-	N/A	2	3
Non-infected	-	-	-	-	-	768	559
Non-infected	-	-	-	-	+	12	2
Non-infected	-	-	-	+	-	4	3
Non-infected	-	-	-	+	+	3	0
Total						883	599

N/A = Specimen not obtained or available for testing
Symp = Symptomatic
Asymp = Asymptomatic

Table 11: *N. gonorrhoeae* Specimen Analysis for Male Patient Infected Status

Infected Status	Amp 1 Urine	Amp 1 Swab	Culture Swab	Combo 2 Urine	Combo 2 Swab	Symp	Asymp
Infected	N/A	+	+	+	+	1	0
Infected	-	N/A	+	N/A	+	0	1
Infected	-	N/A	+	+	+	1	0
Infected	-	-	+	-	-	1	0
Infected	-	+	+	+	+	4	1
Infected	+	N/A	+	N/A	+	0	1
Infected	+	N/A	+	+	N/A	8	0
Infected	+	N/A	+	+	-	1	0
Infected	+	N/A	+	+	+	50	1
Infected	+	-	+	+	+	4	1
Infected	+	+	N/A	+	+	1	0
Infected	+	+	-	+	+	11	1
Infected	+	+	+	-	-	1	0
Infected	+	+	+	-	+	3	0
Infected	+	+	+	+	N/A	1	0
Infected	+	+	+	+	+	229	9
Non-infected	-	-	-	N/A	-	0	1
Non-infected	-	-	-	N/A	+	0	1
Non-infected	-	-	-	-	N/A	17	9
Non-infected	-	-	-	-	-	411	349
Non-infected	-	-	-	-	+	5	10
Non-infected	-	-	-	+	-	1	1
Non-infected	-	-	-	+	+	0	1
Total						750	387

N/A = Specimen not obtained or available for testing
Symp = Symptomatic
Asymp = Asymptomatic

Table 12: Agreement of the APTIMA Combo 2 Assay Results with Infected Patient Status Including Inconclusive Results[1]

Gender	Specimen	Symptoms	CT+/GC+ (# Combo / # Infected) (95% C.I.)	CT+/GC- (# Combo / # Infected) (95% C.I.)	CT-/GC+ (# Combo / # Infected) (95% C.I.)	CT-/GC- (# Combo / #Tested) (95% C.I.)
Male	Swab	Symptomatic	77/81 (95.1%) (87.8 - 98.6)	111/115 (96.5%) (91.3 - 99.1)	199/212 (93.9%) (89.8 - 96.7)	355/378 (93.9%) (91.0 - 96.1)
		Asymptomatic	4/7 (57.1%) (18.4 - 90.1)	61/67 (91.0%) (81.6 - 96.6)	6/6 (100%) (54.1 - 100)	306/319 (95.9%) (93.1 - 97.8)
		All	81/88 (92.0%) (84.3 - 96.7)	172/182 (94.5%) (90.1 - 97.3)	205/218 (94.0%) (90.0 - 96.8)	662/698 (94.8%) (92.9 - 96.4)
	Urine	Symptomatic	78/81 (96.3%) (89.6 - 99.2)	112/115 (97.4%) (92.6 - 99.5)	199/212 (93.9%) (89.8 - 96.7)	369/378 (97.6%) (95.5 - 98.9)
		Asymptomatic	7/7 (100%) (59.0 - 100)	62/67 (92.5%) (83.4 - 97.5)	6/6 (100%) (54.1 - 100)	312/319 (97.8%) (95.5 - 99.1)
		All	85/88 (96.6%) (90.4 - 99.3)	174/182 (95.6%) (91.5 - 98.1)	205/218 (94.0%) (90.0 - 96.8)	682/698 (97.7%) (96.3 - 98.7)
Female	Urine	Symptomatic	37/42 (88.1%) (74.4 - 96.0)	92/102 (90.2%) (82.7 - 95.2)	48/52 (92.3%) (81.5 - 97.9)	707/731 (96.7%) (95.2 - 97.9)
		Asymptomatic	13/14 (92.9%) (66.1 - 99.8)	44/47 (93.6%) (82.5 - 98.7)	15/18 (83.3%) (58.6 - 96.4)	530/540 (98.1%) (96.6 - 99.1)
		All	51/57 (89.5%) (78.5 - 96.0)	136/149 (91.3%) (85.6 - 95.3)	63/70 (90.0%) (80.5 - 95.9)	1238/1272 (97.3%) (96.3 - 98.1)
	Swab	Symptomatic	40/42 (95.2%) (83.8 - 99.4)	88/102 (86.3%) (78.1 - 92.3)	49/52 (94.2%) (84.1 - 98.8)	685/731 (93.7%) (91.7 - 95.4)
		Asymptomatic	14/14 (100%) (76.8 - 100)	45/47 (95.7%) (85.5 - 99.5)	17/18 (94.4%) (72.8 - 99.9)	529/540 (98.0%) (96.4 - 99.0)
		All	55/57 (96.5%) (87.9 - 99.6)	133/149 (89.3%) (83.2 - 93.7)	66/70 (94.3%) (86.0 - 98.4)	1215/1272 (95.5%) (94.2 - 96.6)
Total	Swab	Symptomatic	117/123 (95.1%) (90.0 - 98.2)	199/217 (91.7%) (87.2 - 95.0)	248/264 (93.9%) (90.4 - 96.5)	1040/1109 (93.8%) (92.2 - 95.1)
		Asymptomatic	18/21 (85.7%) (63.7 - 97.0)	106/114 (93.0%) (86.7 - 97.0)	23/24 (95.8%) (78.9 - 100)	835/859 (97.2%) (95.9 - 98.2)
		All	136/145 (93.8%) (88.6 - 97.1)	305/331 (92.1%) (88.7 - 94.8)	271/288 (94.1%) (90.7 - 96.5)	1877/1970 (95.3%) (94.3 - 96.2)
	Urine	Symptomatic	115/123 (93.5%) (87.6 - 97.2)	204/217 (94.0%) (90.0 - 96.8)	247/264 (93.6%) (89.9 - 96.2)	1076/1109 (97.0%) (95.9 - 98.0)
		Asymptomatic	20/21 (95.2%) (76.2 - 99.9)	106/114 (93.0%) (86.7 - 96.9)	21/24 (87.5%) (67.6 - 97.4)	842/859 (98.0%) (96.9 - 98.9)
		All	136/145 (93.8%) (88.6 - 97.1)	310/331 (93.7%) (90.5 - 96.0)	268/288 (93.1%) (89.5 - 95.7)	1920/1970 (97.5%) (96.7 - 98.1)

[1] In this table, results originally classified as inconclusive due to an insufficient number of specimens, single positive results, or missing comparator assay results were reclassified as non-infected and added to the overall database for analysis.

A summary of the APTIMA Positive Control CT/Negative Control GC and APTIMA Positive Control GC/Negative Control CT performance during the clinical trial is presented in Table 13.

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

Control	Statistics	Total RLU (x1000)
Positive, CT/Negative, GC	Maximum	1572
	75th Percentile	1160
	Median	1063
	25th Percentile	996
	Minimum	274
Positive, GC/Negative, CT	Maximum	1359
	75th Percentile	1202
	Median	1093
	25th Percentile	989
	Minimum	167

Precision

Precision testing was performed at three laboratories to obtain measures of repeatability and reproducibility. Each site was provided with three identical panels of 13 samples containing 0 to 500 fg of *C. trachomatis* rRNA, 0 to 25,000 fg of *N. gonorrhoeae* rRNA, or combinations of both *C. trachomatis* and *N. gonorrhoeae* rRNA. Testing was performed over three days using a different kit lot each day. The overall RLU, intra-run, inter-run, and inter-site descriptive statistics are shown in Table 14. Reproducibility was established by spiking swab transport medium and urine transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined.

Table 14: APTIMA Combo 2 Assay Precision Data

			Intra-Run		Inter-Run		Inter-Site	
Panel Member	N	Mean RLU (x 1,000)	SD (RLU)	CV (%)	SD (RLU)	CV (%)	SD (RLU)	CV (%)
High CT Swab	54	1,055	76,588	7.3	83,711	7.9	150,332	14.2
High Dual Swab*	54	2,338	93,449	4.0	90,317	3.9	142,898	6.1
High Dual Urine*	54	2,281	91,487	4.0	106,715	4.7	152,747	6.7
High GC Swab	54	1,265	30,561	2.4	55,642	4.4	34,413	2.7
Mid CT Swab	54	1,001	69,831	7.0	77,701	7.8	159,774	16.0
Mid Dual Swab*	54	2,241	152,377	6.8	58,353	2.6	139,983	6.2
Mid GC Swab	54	1,249	35,142	2.8	60,638	4.9	46,364	3.7
Low CT Swab	54	1,013	61,795	6.1	90,906	9.0	131,207	13.0
Low Dual Swab*	54	2,085	286,034	13.7	161,764	7.8	58,837	2.8
Low Dual Urine*	54	2,201	95,705	4.3	118,760	5.4	106,802	4.9
Low GC Swab	54	1,177	42,478	3.6	69,821	5.9	29,836	2.5
Negative Swab	54	7	1,301	18.3	2,311	32.5	1,901	26.8
Negative Urine	54	7	861	12.0	2,299	32.1	1,994	27.9

*Dual positive panel members contained both *C. trachomatis* and *N. gonorrhoeae* rRNA.

Analytical Sensitivity

Chlamydia trachomatis analytical sensitivity (limits of detection) was determined by directly comparing dilutions of *C. trachomatis* organisms in cell culture and in the assay. The analytical sensitivity claim for the assay is one Inclusion-Forming Unit (IFU) per assay (7.25 IFU/swab, 5 IFU/mL urine) for all 15 *C. trachomatis* serovars. However, dilutions of less than one IFU/assay of all serovars tested positive in the APTIMA Combo 2 Assay.

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

Analytical Specificity

A total of 154 culture isolates were evaluated using the APTIMA Combo 2 Assay. These isolates included 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, parasites, and viruses. All organisms except *C. psittaci, C. pneumoniae*, and the viruses were tested at $1.0x10^6$ cells/assay in both swab and urine transport medium. *C. psittaci* and *C. pneumoniae* were tested at $1.0x10^5$ IFU/assay. The viruses were tested as follows: (a) herpes simplex viruses I and II: $6.0x10^4$ $TCID_{50}$/assay, (b) human papilloma virus 16: $2.9x10^6$ DNA copies/assay and (c) cytomegalovirus: $1x10^6$ infected cell culture cells/assay. Only *C. trachomatis* and *N. gonorrhoeae* samples produced positive results in the APTIMA Combo 2 Assay. The list of organisms tested is shown in Table 15.

Table 15: Analytical Specificity

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

Interfering Substances

Table 16 lists the commonly encountered substances found in swab and/or urine specimens that were tested in the assay. All were tested for potential assay interference in the absence and presence of *C. trachomatis* and *N. gonorrhoeae* at the estimated rRNA equivalent of one *C. trachomatis* IFU/assay (5 fg/assay) and 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.

Table 16:

Interfering Substances Testing

Swab	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

No interference was observed with any of the tested substances.

Recovery

Escherichia coli and *Gardnerella vaginalis* (2.4 x 10^5 cells/assay) were added to samples containing the rRNA equivalent of approximately one *C. trachomatis* IFU (5 fg) and 50 *N. gonorrhoeae* cells (250 fg). These additions did not interfere with the amplification and detection of *C. trachomatis* or *N. gonorrhoeae* rRNA using the APTIMA Combo 2 Assay.

Swab and Urine Specimen Stability Studies

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

Data to support the recommended shipping and storage conditions for urine samples were generated with 10 female and 10 male negative urine samples. The urine samples were spiked with *C. trachomatis* and *N. gonorrhoeae* at final concentrations of 10 IFU and 100 CFU per reaction, respectively. Two sets of the spiked urine samples were held at 4°C and 30°C for 24 hours prior to being added to the Urine Transport Media (UTM). The two sets of UTM samples then were held at 4°C and 30°C, and tested in triplicate at days 0, 1, 5, 20, and 35. All samples were positive for both *C. trachomatis* and *N. gonorrhoeae* when the urine samples were held at 4°C prior to addition of the UTM. When the urine samples were held at 30°C prior to addition of the UTM, all of the samples were positive for *C. trachomatis* and 95% of the samples were positive for *N. gonorrhoeae* at Day 35. These same samples were tested after 116 days of storage at -20°C and -70°C. All samples were positive for both *C. trachomatis* and *N. gonorrhoeae* under both storage conditions.

TIGRIS DTS SYSTEM AGREEMENT DATA

CLINICAL AGREEMENT STUDIES

Agreement of the APTIMA Combo 2 Assay on the fully-automated TIGRIS DTS System with that of the semi-automated DTS System was evaluated with specimens from subjects enrolled at seven geographically diverse clinical sites with low to high prevalence for CT and GC. Two agreement studies were completed. One, the clinical specimen agreement study, tested clinical specimens. The second, the CT/GC clinical panel agreement study, utilized clinical specimens that were screened with the APTIMA Combo 2 Assay on the DTS System. Negative samples were pooled and used to make clinical panels spiked with various concentrations of CT and/or GC. For each of the two agreement studies, the populations evaluated included male and female subjects attending sexually transmitted disease (STD), urgent care, public health, and family planning clinics. Each of the clinical specimens and CT/GC clinical panels was tested individually with the APTIMA Combo 2 Assay on both the TIGRIS DTS and DTS Systems at Gen-Probe.

Clinical Specimen Agreement Study

The clinical specimen agreement study evaluated agreement between the two systems using swab and urine specimens from 485 male and 576 female subjects. Of the 1,991 specimens tested, there were a small percentage that initially tested invalid or equivocal for CT or GC on the TIGRIS DTS System (20, 1.0%) and on the DTS System (14, 0.7%). Upon repeat testing, there were two (2) clinical specimens with equivocal *N. gonorrhoeae* results on the TIGRIS DTS System, which are not included in equivalence calculations. Overall percent agreement and percent positive and negative agreements were calculated. Specimens yielding discordant results between the DTS and TIGRIS DTS Systems were tested in alternate TMA amplification assays for CT and GC, which are nucleic acid amplification tests (NAATs) that target CT or GC rRNA sequences that differ from those targeted in the APTIMA Combo 2 Assay. APTIMA Combo 2 Assay repeat testing on the DTS System was also conducted on specimens yielding discordant TIGRIS DTS System and DTS System results.

Tables 17 and 18 show the overall percent agreements for all paired test results obtained on the TIGRIS DTS and DTS Systems for swab and urine specimens, respectively. Overall agreements were 98.3% for swab specimens and 99.2% for urine specimens. Positive and negative percent agreements are shown in Tables 19 and 20 where results are stratified by symptom status and gender for each specimen type. Positive agreements for the total number of swab and urine specimens tested ranged from 91.4% to 94.3% for CT and 98.0% to 98.8% for GC. Negative agreements for the total number of swab and urine specimens ranged from 99.8% to 99.9% for CT and were 100% for GC. Positive and negative agreements calculated by gender and symptom status were based on small sample sizes and are not intended to represent assay performance. Percentages calculated for each gender and symptom status show agreement of TIGRIS DTS results with the APTIMA Combo 2 semi-automated method. Refer to Tables 4 and 8 for APTIMA Combo 2 performance estimates. Performance estimates would be expected to be similar given the agreement findings across different genders and specimen types.

The somewhat lower percent positive agreements for swab (91.4%) and urine (94.3%) CT results from the TIGRIS DTS versus DTS Systems (Table 19) are due to a small number of additional positives that occurred with the DTS System. As shown in Table 21, the alternate TMA amplification testing results and the results from repeat APTIMA Combo 2 Assay testing on the DTS System indicate that 19 out of the 23 specimens (83%) initially testing negative on the TIGRIS DTS System but positive on the DTS System agreed with TIGRIS DTS results. For CT, alternate TMA amplification results were in 75% (9/12) and 88% (7/8) agreement with TIGRIS DTS System results for swab and urine specimens respectively. For GC, alternate TMA amplification results were in 100% (4/4) agreement with TIGRIS DTS System results for all specimen types. Repeat APTIMA Combo 2 Assay testing on the DTS System of specimens testing initially DTS System positive, but TIGRIS DTS System negative revealed similar findings (see Table 21). Repeat APTIMA Combo 2 Assay DTS System CT results were in 92% (11/12) agreement with the TIGRIS DTS System for swabs and 88% (7/8) agreement for urine specimens. Furthermore, repeat APTIMA Combo 2 Assay DTS System GC results were in 100% (4/4) agreement with the TIGRIS DTS System for all specimen types. Alternate CT TMA amplification testing of the three specimens with TIGRIS DTS System positive, but DTS System negative results, revealed target in one specimen; the same specimen also was negative in repeat DTS System testing. There were no specimens testing TIGRIS DTS GC positive that were not also positive on the DTS System.

CT/GC Clinical Panel Agreement Study

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

Table 22 shows the percent agreements with expected CT and GC results for the TIGRIS DTS System and for the DTS System for each of the 13 CT/GC panels. The concentrations ranged from 10 fold below to 1000 fold above the APTIMA Combo 2 Assay analytical claim limits of 1 IFU/assay for CT and 50 CFU/assay for GC. Also shown in Table 22 is the overall percent agreement (99.3%) between CT/GC panel results from the TIGRIS DTS System and from the DTS System. Positive and negative agreements are shown in Tables 23 and 24 for CT and GC panel results, respectively. For swab and urine panels, positive agreements were 100% and 96.2% respectively for CT, and were both 100% for GC. Swab and urine negative agreements were 100% and 98.0%, respectively, for CT, and were both 100% for GC. Three of 5 female urine panel replicates, which were one log below the APTIMA Combo 2 Assay analytical sensitivity claim of 1 IFU/assay for CT, were CT- on the TIGRIS System. One of 5 female urine panel replicates from a separate panel was CT- on the DTS System.

Table 17. Clinical Specimen Agreement Study: Swab Specimen Results [1]

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

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

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

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

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

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

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

Table 18. Clinical Specimen Agreement Study: Urine Specimen Results Agreement

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

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

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

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

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

Table 19. Clinical Specimen Agreement Study: CT Results

Gender	Specimen	Symptom	N	DTS+ TIGRIS+ n	DTS+ TIGRIS- [1] n	DTS- TIGRIS+ n	DTS- TIGRIS- n	Positive Agreement (95% C.I.)	Negative Agreement (95% C.I.)
All	Swab	Symptomatic	425	84	3[2]	0	338	96.6 (90.3-99.3)	100 (98.9-100)
		Asymptomatic	596	54	10[3]	2[6]	530	84.4 (73.1-92.2)	99.6 (98.6-100)
		All	1021	138	13	2	868	91.4 (85.7-95.3)	99.8 (99.2-100)
	Urine	Symptomatic	407	80	4[4]	0	323	95.2 (88.3-98.7)	100 (98.9-100)
		Asymptomatic	563	52	4[5]	1[7]	506	92.9 (82.7-98.0)	99.8 (98.9-100)
		All	970	132	8	1	829	94.3 (89.1-97.5)	99.9 (99.3-100)
Female[8]	Swab	All	567	58	9	1	499	86.6 (76.0-93.7)	99.8 (98.9- 100)
	Urine	All	562	56	8	1	497	87.5 (76.8-94.4)	99.8 (98.9- 100)
Male[8]	Swab	All	454	80	4	1	369	95.2 (88.3-98.7)	99.7 (98.5- 100)
	Urine	All	408	76	0	0	332	100 (95.3- 100)	100 (98.9- 100)

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

[1] Repeat APTIMA Combo 2 Assay testing on the DTS System indicates that 18 out of the 20 specimens (90%) that were re-tested, initially testing negative on the TIGRIS DTS System but positive on the DTS System, agreed with TIGRIS DTS results. One specimen was not re-tested.
[2] 2/3 were CT- when retested in the APTIMA Combo 2 Assay on the DTS System and were CT- when tested in the alternate TMA amplification assay.
[3] 9/10 were retested. 9/9 were CT- when retested in the APTIMA Combo 2 Assay on the DTS System and 7/9 were CT- in the alternate TMA amplification assay.
[4] 4/4 were CT- when retested in the APTIMA Combo 2 Assay on the DTS System and were CT- in the alternate TMA amplification assay.
[5] 3/4 were CT- when retested in the APTIMA Combo 2 Assay on the DTS System and were CT- in the alternate TMA amplification assay.
[6] 2/2 were CT- when retested in the APTIMA Combo 2 Assay on the DTS System and were CT- in the alternate TMA amplification assay.
[7] 1/1 was CT- when retested in the APTIMA Combo 2 Assay on the DTS System and was CT+ in the alternate TMA amplification assay.
[8] Percentages calculated for each gender show agreement of TIGRIS DTS results with the APTIMA Combo 2 semi-automated method. Refer to Tables 4 and 8 for APTIMA Combo 2 performance estimates. Performance estimates would be expected to be similar given the agreement findings across different genders.

Table 20. Clinical Specimen Agreement Study: GC Results [1]

Gender	Specimen	Symptom	N	DTS+ TIGRIS+ n	DTS+ TIGRIS- n	DTS- TIGRIS+ n	DTS- TIGRIS- n	Positive Agreement (95% C.I.)	Negative Agreement (95% C.I.)
All	Swab	Symptomatic	423	80	1[2]	0	342	98.8 (93.3-100)	100 (98.9-100)
		Asymptomatic	595	18	1[3]	0	576	94.7 (74.0-99.9)	100 (99.4-100)
		All	1018	98	2	0	918	98.0 (93.0-99.8)	100 (99.6-100)
	Urine	Symptomatic	407	72	1[4]	0	334	98.6 (92.6-100)	100 (98.9-100)
		Asymptomatic	563	12	0	0	551	100 (73.5-100)	100 (99.3-100)
		All	970	84	1	0	885	98.8 (93.6-100)	100 (99.6-100)
Female[5]	Swab	All	567	26	1	0	540	96.3 (81.0-99.9)	100 (99.3- 100)
	Urine	All	562	20	1	0	541	95.2 (76.2-99.9)	100 (99.3- 100)
Male[5]	Swab	All	451	72	1	0	378	98.6 (92.6- 100)	100 (99.0- 100)
	Urine	All	408	64	0	0	344	100 (94.4- 100)	100 (98.9- 100)

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

[1]Data not shown: Two male urethral swab specimens tested equivocal in the APTIMA Combo 2 Assay on both the TIGRIS and DTS Systems. One male urethral swab specimen tested GC- in the APTIMA Combo 2 Assay on the TIGRIS System, but equivocal on the DTS System. When retested on the DTS System, this specimen tested GC- in the APTIMA Combo 2 Assay and tested GC- in the alternate TMA amplification assay.

[2,3,4] 1/1 was GC- when retested in the APTIMA Combo 2 Assay on the DTS System and was GC- in the alternate TMA amplification assay.

[5] Percentages calculated for each gender show agreement of TIGRIS DTS results with the APTIMA Combo 2 semi-automated method. Refer to Tables 4 and 8 for APTIMA Combo 2 performance estimates. Performance estimates would be expected to be similar given the agreement findings across different genders.

Table 21. Alternate TMA Amplification and Repeat APTIMA Combo 2 Assay CT and GC Results

		Initial Results		Alternate TMA Amplification Results					Repeat APTIMA Combo 2 Assay DTS Results			
Analyte	**Specimen**	**DTS**	**TIGRIS DTS**	**N**	**# Tested**	**P**	**N**	**% Agrmt TIGRIS**	**# Tested**	**P**	**N**	**% Agrmt TIGRIS**
CT	Swab	P	N	13	12	3	9	75.0%	12	1	11	91.7%
		N	P	2	2	0	2	0.0 %	2	0	2	0.0%
	Urine	P	N	8	8	1	7	87.5%	8	1	7	87.5%
		N	P	1	1	1	0	100%	1	0	1	0.0%
GC	Swab	P	N	2	2	0	2	100%	2	0	2	100%
		N	P	0	n/a			n/a	n/a			n/a
		E	N	1	1	0	1	100%	1	0	1	100%
	Urine	P	N	1	1	0	1	100%	1	0	1	100%
		N	P	0	n/a			n/a	n/a			n/a

P = Positive, N = Negative, E = Equivocal, % Agrmt TIGRIS = Percent agreement with TIGRIS, n/a = Not applicable

Table 22. CT/GC Clinical Panel Agreement Study: Agreement with Expected CT and GC Results

Panel Member CT/GC	Panel Member Concentration[1]		Replicates	CT		GC	
	CT IFU/mL	GC CFU/mL		TIGRIS %Agrmt	DTS %Agrmt	TIGRIS %Agrmt	DTS %Agrmt
Low/Low	2.5	125	20	100	100	100	100
Low/High	2.5	125,000	20	100	95[3]	100	100
High/Low	2,500	125	20	100	100	100	100
High/High	2,500	125,000	20	100	100	100	100
Very Low/Neg	0.25[2]	0	20	85[4]	100	100	100
Low/Neg	2.5	0	20	100	100	100	100
Medium/Neg	25	0	20	100	100	100	100
High/Neg	2,500	0	20	100	100	100	100
Neg/Very Low	0	12.5	20	100	100	100	100
Neg/Low	0	125	20	100	100	100	100
Neg/Medium	0	1,250	19	100	100	100	100
Neg/High	0	125,000	20	100	100	100	100
Neg/Neg	0	0	20	100	100	100	100

Overall Percent Agreement between TIGRIS and DTS (95% C.I.): 99.3% (98.3% - 99.8%)

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

[1]A collection tube contains approximately 2.9 mL of transport medium for swab specimens and 4.0 mL of transport medium/urine mixture for urine specimens.
[2]The CT concentration in this CT/GC clinical panel member is one log below the APTIMA Combo 2 Assay analytical sensitivity claim of 1 IFU/assay (7.25 IFU/swab, 5 IFU/mL urine).
[3]One of 5 female urine panel replicates was CT- on the DTS System.
[4]Three of 5 female urine panel replicates were CT- on the TIGRIS System.

Table 23. CT/GC Clinical Panel Agreement Study: CT Results

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

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

[1] Three of 5 female urine panel replicates, which were one log below the APTIMA Combo 2 Assay analytical sensitivity claim of 1 IFU/assay for CT, were CT- on the TIGRIS System.
[2] One of 5 female urine panel replicates was CT- on the DTS System.

Table 24. CT/GC Clinical Panel Agreement Study: GC Results

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

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

A summary of the APTIMA Positive Control, CT/Negative Control, GC and APTIMA Positive Control, GC/Negative Control, CT performance during the clinical agreement studies is presented in Table 25.

Table 25: Distribution of Total RLU of the APTIMA Assay Controls

Control	Statistics	Total RLU (x1000)
Positive, CT/Negative, GC	Maximum	1210
	75th Percentile	1094
	Median	1043.5
	25th Percentile	986
	Minimum	906
Positive, GC/Negative, CT	Maximum	1204
	75th Percentile	1143
	Median	1081.5
	25th Percentile	1030
	Minimum	913

PRECISION

TIGRIS DTS System precision (i.e., reproducibility) was evaluated at one external clinical site and at Gen-Probe. APTIMA Combo 2 Assay precision was evaluated across three TIGRIS Systems, two study sites, two APTIMA Combo 2 Assay kit lots and four operators. Table 26 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.

At the external site, two operators performed three worklists (i.e., runs) per APTIMA Combo 2 Assay kit lot on one TIGRIS DTS System, completing a total of 6 worklists each. At Gen-Probe, two operators performed three worklists per APTIMA Combo 2 Assay kit lot on each of two TIGRIS DTS Systems, completing a total of 12 worklists each. Thus, a total of 36 worklists were completed overall. Each worklist was composed of six identical, 12-member precision panels containing 0 to 2,000 fg/assay of CT rRNA and/or 0 to 2,433 fg/assay of GC rRNA. Each worklist was composed of six identical, 12-member precision panels containing 0 to 2,000 fg/assay of CT rRNA and/or 0 to 5,000 fg/assay of GC rRNA. Panel members containing CT and GC were categorized as having low (5 or 100 fg/assay), mid (1000 fg/assay), or high (≥ 2000 fg/assay) concentrations of CT and as having low (≤ 250 fg/assay), mid (approx. 2400 fg/assay), or high (5000 fg/assay) concentrations of GC. Reproducibility was established by spiking swab transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Precision was estimated according to NCCLS Guidelines (NCCLS document EP5-A, 1999).

Table 26. TIGRIS DTS System Precision Data

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

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

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with standard deviation and %CV is set to 0. See NCCLS Approved Guidelines EP5-A, 1999.

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

TIGRIS DTS SYSTEM ANALYTICAL PERFORMANCE

Analytical Sensitivity Equivalence Study

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

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

*A*nalytical sensitivity for both *C. trachomatis* and *N. gonorrhoeae* on the TIGRIS DTS System is equivalent to that on the DTS System.

Analytical Specificity Equivalence Study

For a nucleic acid amplification assay, analytical specificity with respect to individual organisms is largely determined by the chemistry of the assay (e.g. oligonucleotide sequences) rather than by the platform. Because the reagents for the APTIMA Combo 2 assay are identical between the TIGRIS DTS System and the DTS System, analytical specificity experiments on the TIGRIS DTS System were designed to focus on the most challenging culture isolates. These organisms included those known to cross-react in other amplification assays. Twenty-four (24) culture isolates were selected from the panel of organisms in Table 15, including 3 organisms that are most closely related to *C. trachomatis* and 17 organisms that are most closely related to *N. gonorrhoeae*. All of the organisms tested produced negative results on the TIGRIS DTS System.

Analytical specificity on the TIGRIS DTS System is equivalent to that on the DTS System.

Interfering Substances Equivalence Study

Whole blood, a substance commonly found in urogenital specimens and known to interfere in some amplification assays, was used to establish that the TIGRIS DTS System tolerates similar levels of potentially interfering substances as does the DTS System. Fresh blood was added to clinical swab and urine specimen pools, then tested for potential assay interference in the absence and presence of CT and GC target at the estimated rRNA equivalent of one CT IFU/assay (5 fg/assay) and 50 GC cells/assay (250 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. Specimens were tested on two TIGRIS DTS Systems. All samples containing target nucleic acid were positive when tested at a level of 10% blood in swab specimens and 30% blood in urine specimens. All samples that did not contain target were negative for both CT and GC. These results are identical to those demonstrated for the DTS System (Table 16).

Tolerance of blood in swab and urine specimens on the TIGRIS DTS System is equivalent to that on the DTS System.

Carryover Studies

To establish that the TIGRIS DTS system minimizes the risk of false positive results arising from carryover contamination, a multi-day analytical study was conducted using spiked panels on three TIGRIS DTS Systems. The study used 20% high-target *N. gonorrhoeae* samples containing 1 x 10^9 cells/reaction, which were randomly spaced amongst 80% negative samples containing swab transport media. Over the course of the study, 1,372 high-target samples and 5,516 negative samples were tested across the three TIGRIS DTS Systems. The overall carryover rate, including both false positive and equivocal results, averaged 0.3% (18/5491). A total of 25 negative samples were reported as invalid and were excluded from the calculation. A separate analysis was conducted on a subset of the study population comprised of the negative samples that immediately followed a high-target positive. The carryover rate for this subset of the population, including both false positive and equivocal results, averaged 1.1% (12/1097). For false positives in this subset, the carryover rate ranged from 0% to 1.1% across the three TIGRIS DTS Systems. For equivocals in this subset, the carryover rate ranged from 0% to 0.9% across the three TIGRIS DTS Systems. These results demonstrate that carryover contamination is minimized on the TIGRIS DTS System.

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Gen-Probe Incorporated
San Diego, CA 92121
Customer Service and Technical Support
(858) 410-8000; (800) 523-5001
(800) 342-7441 (in Canada)
www.gen-probe.com

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