APTIMA® Neisseria gonorrhoeae Assay

For *in vitro* diagnostic use.

For US export only.

Intended Use

The APTIMA Neisseria gonorrhoeae Assay is a target amplification nucleic acid probe test that utilizes target capture for the *in vitro* qualitative detection of ribosomal RNA (rRNA) from *Neisseria gonorrhoeae* (GC) to aid in the diagnosis of gonococcal urogenital disease using the TIGRIS DTS Automated Analyzer or semi-automated instrumentation as specified. The assay may be used to test the following specimens from symptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; and female and male urine specimens. The assay may be used to test the following specimens from asymptomatic individuals: clinician-collected endocervical and vaginal swab specimens, patient-collected vaginal swab specimens[1]; and female and male urine specimens. The assay is also intended for use with the testing of gynecological specimens, from both symptomatic and asymptomatic patients, collected in the PreservCyt Solution and processed with the Cytyc ThinPrep 2000 System.

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

Summary and Explanation of the Test

Neisseria gonorrhoeae infections are one of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 336,742 new cases of GC infections were reported to the Centers for Disease Control in 2008 (2).

N. gonorrhoeae is the causative agent of gonorrheal disease. *Neisseria* are non-motile, gram-negative diplococci. The majority of gonorrheal infections are uncomplicated lower genital tract infections and may be asymptomatic. However, if left untreated in women, infections can ascend and cause pelvic inflammatory disease (PID). PID can manifest as endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscesses. A smaller percentage of persons with gonococcal infections may develop Disseminated Gonococcal Infection (DGI) (8, 11).

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

First generation NAATs for GC have technological issues that have limited their performance. These issues include cumbersome specimen processing and specimen inhibition that can yield false negative results (6). The APTIMA Neisseria gonorrhoeae Assay (APTIMA GC Assay) is a second generation NAAT that utilizes target capture, Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (HPA) technologies to streamline specimen processing, amplify target rRNA, and detect amplicon, respectively. Studies comparing performance and specimen inhibition of various amplification systems have demonstrated the benefits of target capture, TMA, and HPA (4, 7).

According to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* 2002 Screening Guidelines, CDC recommends a number of options for follow-up on a positive screening test "if a low positive predictive value can be expected or if a false-positive result would have serious psychosocial or legal consequences" (1). One of these options for additional testing can be a different FDA-cleared nucleic acid amplification test that uses a different target than the initial test. The APTIMA GC Assay and the APTIMA COMBO 2 Assay both target the 16S rRNA subunit for capture and detection. The capture probe is the same for both assays, but the APTIMA GC Assay recognizes a different region of the 16S rRNA subunit than the APTIMA COMBO 2 Assay for detection.

Principles of the Procedure

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

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

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

DTS Systems Reagents

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

Materials Provided

APTIMA Neisseria gonorrhoeae Assay Kit, 100 tests (2 boxes) (Cat. No. 301091)

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

Symbol	Component	Quantity	Description
A	APTIMA Amplification Reagent GC	1 vial	*Primers, dNTPs, NTPs, and co-factors in TRIS buffer with preservative.*
E	APTIMA Enzyme Reagent	1 vial	*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*
P	APTIMA Probe Reagent GC	1 vial	*Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*
TCR-B	APTIMA Target Capture Reagent B	1 x 0.35 mL	*Non-infectious nucleic acid in a buffered solution containing < 5% detergent.*
PGC/ NCT	APTIMA Positive Control, GC/ Negative Control, CT	3 x 1.7 mL	*Non-infectious GC nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 50 GC cells (250 fg/assay*).*
PCT/ NGC	APTIMA Positive Control, CT / Negative Control, GC	3 x 1.7 mL	*Non-infectious CT nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 1 CT IFU (5 fg/assay*).*

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

Also included in the refrigerated box are the following:

Storage Tray (2°C to 30°C):

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

Also included in the refrigerated box are the following:

	Reconstitution Collars	3 each	
	Sealing Cards	1 package	

Non-Refrigerated Box (15°C to 30°C):

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

DTS Systems Materials

Materials Required But Not Provided

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

APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens (Cat. No. 301041)

APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens (Cat. No. 301040)

APTIMA Urine Specimen Transport Tubes for Male and Female Urine Specimens (Cat. No. 105575)

APTIMA Vaginal Swab Specimen Collection Kit (Cat. No. 301162)

APTIMA Specimen Transfer Kit (Cat. No. 301154C)

APTIMA Auto Detect Kit (Cat. No. 301048)

LEADER HC+ Luminometer (Cat. No. 104747-01)

GEN-PROBE Target Capture System (TCS) (Cat. No. 104555)

Incubators and vortexers:

Either:

- 2 Multi-tube vortex mixers (Cat. No. 102160)
- 3 Circulating water baths (62ºC ± 1ºC, 42ºC ± 1ºC, 62ºC ± 1ºC) (Cat. No. 104586)
- 3 Water bath spacers (Cat. No. 104627)

Or:

- 2 SB100 Dry Heat Bath/Vortexers (Cat. No. 105524) (Additional SB100 baths may be required as test volume increases)

2 eppendorf Repeater Plus pipettors (Cat. No. 105725)

2 pipettors, 1000 µL RAININ PR1000 (Cat. No. 901715)

eppendorf pipettor, 20 µL to 200 µL (Cat. No. 105726)

Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL) (Cat. Nos. 21-381-329, 21-381-330, 21-381-115, respectively)

Tips, P1000 Style, special diameter tip only available from Gen-Probe (Cat. No. 105049)

Pipette tips 20 µL to 200 µL (705512 Fisher)

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

Ten Tip Cassettes (TTC) (Cat. No. 104578)

SysCheck calibration standard (Cat. No. 301078)

Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution

Standard urine collection containers, without preservatives

Large-capped plastic container

APTIMA penetrable caps (Cat. No. 105668)

Replacement, non-penetrable caps (Cat. No. 103036A)

Optional Materials

APTIMA Controls Kit (Cat. No. 301110)

GEN-PROBE Bleach Enhancer for Cleaning (Cat. No. 302101) for routine cleaning of surfaces and equipment

STD Proficiency Panel (Cat. No. 102325)

TECAN Freedom EVO 100/4 (Cat. No. 900932)

DTS 800 Systems APTIMA COMBO 2 Deck Plate (Cat. No. 105200)

Tips, 1000 µL conductive, liquid sensing, TECAN 10612513

Reagent reservoir (40 mL quarter module) (Cat. No. 104765)

Split reagent reservoir (19 mL x 2 quarter module) (Cat. No. 104763)

TIGRIS DTS System Reagents

Reagents for the APTIMA GC Assay are provided below for the TIGRIS DTS System. Reagent Identification Symbols are also listed next to the reagent name.

Materials Provided

APTIMA Neisseria gonorrhoeae Assay Kit

250 tests (2 boxes and 1 Controls kit) (Cat. No. 301196)

100 tests (2 boxes and 1 Controls kit) (Cat. No. 303092)

2 x 50 tests (2 boxes and 1 Controls kit) (Cat. No. 302225)

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

Symbol	Component	Quantity		
		250 test kit	100 test kit	2 x 50 test kit
A	**APTIMA Amplification Reagent GC** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial	1 vial	2 vials
E	**APTIMA Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial	1 vial	2 vials
P	**APTIMA Probe Reagent GC** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial	1 vial	2 vials
TCR-B	**APTIMA Target Capture Reagent B** *Non-infectious nucleic acid in a buffered solution containing < 5% detergent.*	1 x 0.61 mL	1 x 0.30 mL	2 x 0.143 mL
	Reconstitution Collars			6

Room Temperature Box (Box 2 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity		
		250 test kit	100 test kit	2 x 50 test kit
AR	**APTIMA Amplification Reconstitution Solution GC** *Aqueous solution containing preservatives.*	1 x 27.7 mL	1 x 11.9 mL	2 x 6.4 mL
ER	**APTIMA Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL	1 x 6.3 mL	2 x 3.3 mL
PR	**APTIMA Probe Reconstitution Solution GC** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL	1 x 15.2 mL	2 x 7.7 mL

Symbol	Component	Quantity		
		250 test kit	100 test kit	2 x 50 test kit
S	**APTIMA Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL	1 x 43.0 mL	2 x 20 mL
TCR	**APTIMA Target Capture Reagent GC** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 mL	1 x 26.0 mL	2 x 17 mL
	Reconstitution Collars	3	3	
	Transfer Pipettes			2
	Master Lot Barcode Sheet	1 sheet	1 sheet	1 sheet

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

Symbol	Component	Quantity
PCT/ NGC	**APTIMA Positive Control, CT / Negative Control, GC** *Non-infectious CT nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 1 CT IFU (5 fg/assay*).*	5 x 1.7 mL
PGC/ NCT	**APTIMA Positive Control, GC / Negative Control, CT** *Non-infectious GC nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 50 GC cells (250 fg/assay*).*	5 x 1.7 mL

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

TIGRIS DTS System Materials

Materials Required but Not Provided

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

APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens (Cat. No. 301041)

APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens (Cat. No. 301040)

APTIMA Urine Specimen Transport Tubes for Male and Female Urine Specimens (Cat. No. 105575)

APTIMA Vaginal Swab Specimen Collection Kit (Cat. No. 301162)

APTIMA Specimen Transfer Kit (Cat. No. 301154C)

TIGRIS DTS System (Cat. No. 105118)

Tips, 1000 µL conductive, liquid sensing, TECAN 10612513

APTIMA Assay Fluids Kit (Cat. No. 302382)
(APTIMA Wash Solution, APTIMA Buffer for Deactivation Fluid, and APTIMA Oil Reagent)

APTIMA System Fluid Preservative Kit (Cat. No. 302380)

APTIMA Auto Detect Kit (Cat. No. 301048)

TIGRIS DTS System Run Kit (Cat. No. 301191) or

Multi-tube Units (MTU) (Cat. No. 104772-02)

MTU/Tiplet Waste Bag Kit (Cat. No. 900907)

MTU Waste Deflectors (Cat. No. 900931)

MTU Waste Covers (Cat. No. 105523)

Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution

Water for the TIGRIS DTS System (consult the TIGRIS DTS System Operator's Manual for specifications)

Disposable gloves

SysCheck calibration standard (Cat. No. 301078)

APTIMA penetrable caps (Cat. No. 105668)

Replacement, non-penetrable caps (Cat. No. 103036A)

Replacement Caps for the 250-test kits

Amplification and Probe reagent reconstitution solutions CL0041 (100 caps)
Enzyme Reagent reconstitution solution 501616 (100 caps)
TCR and Selection reagent CL0040 (100 caps)

Replacement Caps for the 100-test kits

Amplification, Enzyme, and Probe reagent reconstitution solutions CL0041 (100 caps)
TCR and Selection reagent 501604 (100 caps)

Replacement Caps for the 2 x 50-test kits

Amplification, Enzyme, and Probe reagent reconstitution solutions 501603 (100 caps)
TCR and Selection reagent 501604 (100 caps)

Optional Materials

APTIMA Controls Kit (Cat. No. 301110)

GEN-PROBE Bleach Enhancer for Cleaning (Cat. No. 302101) for routine cleaning of surfaces and equipment

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For additional specific warnings, precautions and procedures to control contamination for the TIGRIS DTS System, consult the TIGRIS DTS System Operator's Manual.

Laboratory Related

C. Use only supplied or specified disposable laboratory ware.

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

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

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

DTS Systems Specific

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

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

Specimen Related

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

J. The PreservCyt Solution, a component of the ThinPrep 2000 System, has been validated as an alternative medium for testing with the APTIMA GC Assay. PreservCyt Solution liquid Pap specimens processed using the ThinPrep 3000 Processor or other instruments have not been evaluated to test for *Neisseria gonorrhoeae* using the APTIMA GC Assay.

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. 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.

M. Expiration dates listed on the collection kits pertain to the collection site and not the testing facility. Samples collected any time prior to the expiration date of the collection kit, and transported and stored in accordance with the package insert, are valid for testing even if the expiration date on the collection tube has passed.

N. 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.

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

P. If the lab receives a swab specimen transport tube with no swab, two swabs, a cleaning swab, or a swab not supplied by Gen-Probe, the specimen must be rejected. Prior to rejecting a swab transport tube with no swab, verify that it is not an APTIMA Specimen Transfer Tube as this specimen transport tube will not contain a swab.

Q. For PreservCyt liquid Pap specimens, collect according to the manufacturer's instructions. Aliquots subsequently removed from the PreservCyt vial for testing by the APTIMA GC Assay should be processed using only the APTIMA Specimen Transfer Kit.

R. Upon piercing, liquid can discharge from APTIMA transport tube caps under certain conditions. Follow instructions in the appropriate test procedure to prevent this occurrence.

Assay Related

S. The performance of vaginal swab specimens have not been evaluated in pregnant women.

T. The performance of vaginal swab and PreservCyt liquid Pap specimens have not been evaluated in women less than 16 years of age.

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

V. Do not interchange, mix, or combine assay reagents from kits with different lot numbers. APTIMA controls and assay fluids can be from different lot numbers.

DTS Systems Specific

W. Tips with hydrophobic plugs must be used. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the target capture and amplification steps, and one for use in the HPA steps. Two micropipettors must be dedicated for use in this assay: one for use in specimen transfer and one for use in reagent preparation. All pipettors must be cleaned regularly as described in *Procedural Notes*.

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

Y. Adequate mixing is necessary to achieve accurate assay results. For complete details, see *Procedural Notes*.

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

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

AB. Sealing cards should be disposed of in the waste container immediately after removing them from reaction tubes. Fresh sealing cards should always be used: they should never be re-used from a previous step. Sealing cards should be firmly fixed to the top of all reaction tubes.

Reagent Storage and Handling Requirements

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

- APTIMA Amplification Reagent GC
- APTIMA Enzyme Reagent
- APTIMA Probe Reagent GC
- APTIMA Target Capture Reagent B
- APTIMA Positive Control, GC / Negative Control, CT
- APTIMA Positive Control, CT / Negative Control, GC

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

- APTIMA Amplification Reconstitution Solution GC
- APTIMA Enzyme Reconstitution Solution
- APTIMA Probe Reconstitution Solution GC
- APTIMA Selection Reagent

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

- APTIMA Target Capture Reagent GC
- APTIMA Wash Solution
- APTIMA Buffer for Deactivation Fluid
- APTIMA Oil Reagent

D. Working Target Capture Reagent GC (wTCR GC) is stable for 60 days when stored at 15°C to 30°C. Do not refrigerate.

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

F. Discard any unused reconstituted reagents and wTCR GC after 60 days or after the Master Lot expiration date, whichever comes first.

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

H. Reagents from 250- or 50-test bottles stored on-board the TIGRIS DTS System have 48 hours of on-board stability.

I. Reagents from 100-test bottles stored on-board the TIGRIS DTS System have 96 hours of on-board stability.

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

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

L. **Do not freeze the reagents**.

Specimen Collection and Storage

The APTIMA GC Assay is designed to detect the presence of GC in clinician-collected endocervical, vaginal and male urethral swab specimens, patient-collected vaginal swab specimens, female and male urine specimens, and PreservCyt liquid Pap specimens. Performance with specimens other than those collected with the following specimen collection kits have not been evaluated:

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- APTIMA Urine Collection Kit for Male and Female Urine Specimens
- APTIMA Vaginal Swab Specimen Collection Kit
- APTIMA Specimen Transfer Kit (for use with gynecological samples processed with the Cytyc ThinPrep 2000 System)

A. Instructions for Collection:

Refer to the appropriate specimen collection kit package insert for collection instructions.

B. Specimen transport and storage before testing:

1. Swab specimens:
   a. After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested. Specimens must be assayed with the APTIMA GC Assay within 60 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after collection (see *Specimen Stability Studies*).
2. Urine specimens:
   a. Urine samples that are still in the primary collection container must be transported to the lab at 2°C to 30°C. Transfer the urine sample into the APTIMA urine specimen transport tube within 24 hours of collection. Store at 2°C to 30°C and test within 30 days of collection.
   b. After collection, transport the processed urine specimens in the APTIMA urine specimen transport tube at 2°C to 30°C and store at 2°C to 30°C until tested. Processed urine specimens should be assayed with the APTIMA GC Assay within 30 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after collection (see *Specimen Stability Studies*).
3. PreservCyt Solution liquid Pap specimens:
   a. PreservCyt Solution liquid Pap specimens intended for GC testing must be processed for cytology within 30 days of collection when stored at 2°C to 30°C (see *Specimen Stability Studies*).
   b. Process the PreservCyt Solution liquid Pap specimen in accordance with the *ThinPrep 2000 Processor Operator's Manual* and the APTIMA Specimen Transfer Kit package insert. Transfer 1 mL of the fluid remaining in the PreservCyt Solution vial into an APTIMA Specimen Transfer tube according to the instructions in the APTIMA Specimen Transfer Kit package insert.
   c. Once the PreservCyt Solution liquid Pap specimen is transferred to the APTIMA Specimen Transfer tube, the specimen must be assayed with the APTIMA GC Assay within 30 days when stored at 2°C to 8°C or 14 days when stored at 15°C to 30°C. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after transfer (see *Specimen Stability Studies*).
   d. If the Aliquot Removal procedure will be used, refer to the *ThinPrep 2000 or ThinPrep 3000 Processor Operator's Manual - Addendum* for instructions on aliquot removal.

C. Specimen storage after testing:

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen transport tubes should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable caps and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 RCF (Relative Centrifugal Force) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination**.

Note: Federal requirements for packaging must be met when specimens are transported by common land and air carriers. Refer to 42 CFR, Part 72. The most current requirements may be obtained from the Centers for Disease Control and Prevention Office of Health and Safety (CDC) in Atlanta, Georgia at 1-800-467 4922 or the CDC web site.

DTS Systems Test Procedure

A. Equipment Preparation

1. Adjust one water bath to 62°C ± 1°C (for target capture, and primer annealing), a second water bath to 42°C ± 1°C (for amplification), and a third water bath to 62°C ± 1°C (for HPA). If using the SB100 Dry Heat Bath/Vortexer, refer to the SB100 Application Sheet.
2. Prior to starting the assay, wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the bleach to contact surfaces and pipettors for at least one minute, then follow with a water rinse. Do not allow the bleach to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed, absorbent laboratory bench covers.
3. Place a sufficient number of Ten Tip Cassettes (TTC) into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with APTIMA Wash Solution and the aspiration manifold is connected to the vacuum pump. (Refer to the *Target Capture System Operator's Manual*.)

B. Reagent Reconstitution

Reagent Reconstitution should be performed prior to beginning specimen transfer.

1. To reconstitute the APTIMA Enzyme, Amplification GC, and Probe GC Reagents:
   a. Pair the appropriate reconstitution solution with the lyophilized reagent. The labels are color coded so that they can be paired correctly.
   b. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   c. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
   d. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
   e. Slowly invert the assembled bottle and vial. Allow the solution to drain from the bottle into the vial (Figure 1, Step 3).
   f. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 1, Step 4).
   g. Wait for the lyophilized reagent to go into solution, then invert the assembled bottle and vial again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the bottle.
   h. Remove the reconstitution collar from the bottle (Figure 1, Step 6).
   i. Recap the bottle. Peel and discard the top label. Record operator initials, the reconstitution date, and lyophilized reagent lot number on the remaining label (Figure 1, Step 7).
   j. Discard the reconstitution collar and vial (Figure 1, Step 8).

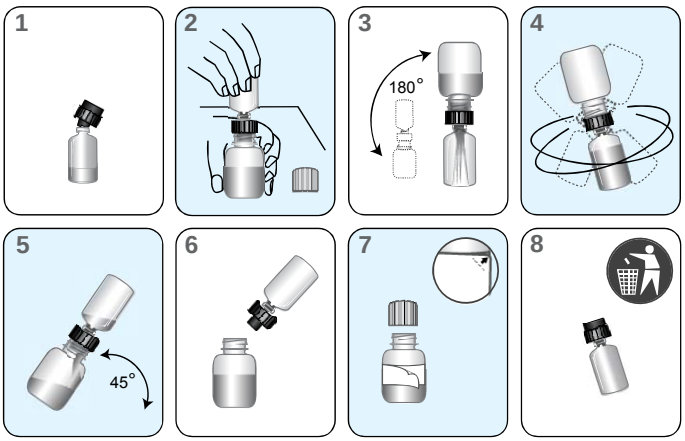

Figure 1. DTS Systems reconstitution process

2. Previously reconstituted Probe GC, Amplification GC, and Enzyme Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay. If Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C for 1 to 2 minutes. After this heat step, the Probe Reconstitution Solution may be used even if residual precipitate remains. After resuspension, mix by gentle inversion.

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

3. Prepare working Target Capture Reagent GC (wTCR GC) as follows:
   a. Transfer 20 mL of TCR GC to an appropriately sized, dedicated, clean, dry container.
   b. Using a micropipettor, add 200 µL of TCR-B into the TCR GC.
   c. Thoroughly mix the solution by swirling.
   d. Label the container. Record operator initials, preparation date, and both lot numbers.

   Note: For a smaller number of reactions (specimens and controls), use the following to calculate volumes of TCR GC and TCR-B:

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

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

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*.

Rack Setup

1. Allow the controls and specimens to reach room temperature prior to processing.
2. **Do not vortex specimens.**
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single blue APTIMA collection swab in a unisex swab specimen transport tube.
   b. The presence of a single pink APTIMA collection swab in a vaginal swab specimen transport tube.
   c. A final volume of urine between the black fill lines of a urine specimen transport tube.
   d. The absence of a swab in the APTIMA specimen transport tube for PreservCyt Solution liquid Pap specimens.
4. Inspect specimen tubes before piercing them:
   a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.
   c. If the liquid level in a urine specimen tube is not between the two black indicator lines on the label, the specimen must be rejected. Do not pierce an overfilled tube.
   d. If a urine specimen tube contains precipitate, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, visually ensure that the precipitate does not prevent delivery of the specimen.

   Note: Failure to follow Steps 4a-c may result in liquid discharge from the transport tube cap.
5. In the Ten Tube Unit (TTU) rack, place enough TTUs to accommodate the controls and specimens.
6. If a worklist is desired, create the worklist at this point. For instructions on creating a worklist, refer to the *APTIMA Assay Software Operator's Manual*.
7. Thoroughly mix the TCR GC plus TCR-B reagent. Using the repeat pipettor, add 100 µL into each reaction tube.

8. **The first tube of the assay must contain the negative control, and the second tube must contain the positive control.**

   a. The negative control label for the APTIMA GC Assay is pink. The label text identifies the negative control as "CONTROL + CT PCT / CONTROL – GC NGC". The positive control label for the APTIMA GC Assay is blue-green. The label text identifies the positive control as "CONTROL + GC PGC / CONTROL – CT NCT." See instructions in Step 8b.

   b. Hold the negative control tube (pink-labeled tube) in one hand or keep it in a rack. Using a micropipettor, pierce the cap, taking care not to drive the tip into the bottom of the tube. Add 400 µL of the negative control to the first reaction tube. In the same manner, add 400 µL of the positive control (blue-green labeled tube) to the second reaction tube.

9. Continue the rack setup procedure by adding 400 µL of each specimen into the remaining reaction tubes. Use a new pipette tip for each specimen and control. The acceptable volume of control or specimen added to a reaction tube is 400 µL ± 100 µL. See *Control and Specimen Pipetting* in *Procedural Notes*.

10. If specimens with standard caps (non-penetrable caps) are to be tested, they must be centrifuged for 5 minutes at 420 RCF (Relative Centrifugal Force) to bring all of the liquid down to the bottom of the tube before uncapping. **Avoid splashing and cross-contamination.**

Target Capture

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

11. Cover the TTUs with sealing cards and shake the rack gently by hand. **Do not vortex.** Incubate the rack at 62°C ± 1°C in a water bath for 30 ± 5 minutes.

12. Remove the rack from the water bath and blot the bottoms of the tubes dry on absorbent material.

13. Ensure the sealing cards are firmly seated. If necessary, replace them with new sealing cards and seal the TTUs tightly.

14. Vortex the rack for 60 seconds on the multi-tube vortex mixer. See *Vortexing* in *Procedural Notes*. Begin vortexing within 2 minutes of removal of the rack from the water bath.

15. Without removing the sealing cards, incubate the rack at room temperature for 30 ± 5 minutes.

16. Place the rack on the TCS magnetic base for 5 to 10 minutes.

17. Prime the dispense station pump line 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 that all ten nozzles are delivering a steady stream of liquid.

18. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the aspiration manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification.[1] It may take 15 seconds to achieve this reading. Reconnect the aspiration manifold, and ensure that the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed and the aspiration manifold tubing is dry.

19. 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.

20. After the aspiration is complete, eject the tips into their original TTC. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each specimen.

21. 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.

22. Cover the tubes with a sealing card and remove the rack from the TCS magnetic base. Vortex the rack once on the multi-tube vortex mixer. See *Vortexing* in *Procedural Notes*.

23. Place the rack on the TCS magnetic base for 5 to 10 minutes.

24. Aspirate all liquid as in Steps 19 and 20.

25. After the final aspiration, remove the rack from the TCS magnetic base and visually inspect the tubes to ensure that all liquid has been aspirated and all tubes contain magnetic particle pellets. If any liquid is visible, place the rack back onto the TCS magnetic base for 2 minutes and repeat the aspiration for that TTU using the same tips used previously for each specimen.

    Note: If a magnetic particle pellet is visible after aspiration is completed, the tube may be accepted. If no pellet is visible, the specimen should be retested. If the same specimen does not contain a magnetic particle pellet at this step in a subsequent run, this may indicate a specimen-specific problem. Re-collection of the specimen is recommended in this situation.

D. Amplification

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

1. Using the repeat pipettor, add 75 µL of the reconstituted Amplification Reagent GC to each reaction tube. All reaction mixtures in the rack should now be red.

2. Using the repeat pipettor, add 200 µL of Oil Reagent to each reaction tube.

3. Cover the tubes with a sealing card and vortex them on the multi-tube vortex mixer.

4. Incubate the rack in a water bath at 62°C ± 1°C for 10 ± 5 minutes.

5. Transfer the rack into a water bath at 42°C ± 1°C and incubate 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 reaction tube. All reaction mixtures should now be orange.

7. Immediately cover the tubes with a fresh sealing card, remove the rack from the water bath, and mix the reaction tubes by gently shaking the rack by hand.

8. Incubate the rack in a water bath at 42°C ± 1°C for 60 ± 15 minutes.

E. Hybridization Protection Assay (HPA)

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

The repeat pipettor used in the hybridization and selection steps should be dedicated for use in these steps only. See *Warnings and Precautions.*

1. Hybridization

   a. Remove the rack from the water bath and transfer it to the HPA area. Using the repeat pipettor, add 100 µL of the reconstituted Probe Reagent GC to each reaction tube. All reaction mixtures should now be yellow.

1 See the Target Capture System Vacuum Specifications Sheet located at the back of the *Target Capture System Operator's Manual* or contact Technical Support.

b. Cover the tubes with a sealing card and vortex the rack on the multi-tube vortex mixer.

c. Incubate the rack in a 62°C ± 1°C water bath for 20 ± 5 minutes.

d. Remove the rack from the water bath and incubate it at room temperature for 5 ± 1 minutes.

2. Selection

a. Using the repeat pipettor, add 250 µL of Selection Reagent to each reaction tube. All reaction mixtures should now be red.

b. Cover the tubes with a sealing card, vortex the rack for 10 seconds or until the color is uniform, and incubate the rack in a water bath at 62°C ± 1°C for 10 ± 1 minutes.

c. Remove the rack from the water bath.

3. Detection

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

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

Note: This temperature range is critical for assay performance.

b. For use of the LEADER HC+ Luminometer and the APTIMA Assay Software refer to the *LEADER HC+ Luminometer Operator's Manual* and the *APTIMA Assay Software Operator's Manual.*

c. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the tests.

d. Prepare the LEADER HC+ Luminometer by placing one empty TTU in cassette position number one and performing the **Wash** protocol.

e. Load the TTUs into the luminometer.

f. Log on to the computer. Click on **New Run,** choose **APTIMA GC Assay Protocol** and enter the number of tubes (controls and specimens). Click **Next** to begin the run.

Note: The run must be completed within 2 hours of the end of the selection step incubation.

g. Prepare Deactivation Fluid by mixing equal volumes of 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution and APTIMA Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. Deactivation Fluid is stable for 4 weeks at room temperature. Discard Deactivation Fluid after 4 weeks or after 100 processed samples have been deactivated (whichever comes first).

h. After removing the used TTUs from the luminometer, place the TTUs into the container of Deactivation Fluid. 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.

Procedural Notes

A. Controls

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

B. Control and Specimen Pipetting

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

C. Reagents

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

D. Temperature

1. The target capture, amplification, hybridization, and selection steps are temperature dependent. Therefore, it is imperative that the water baths be maintained within their specified temperature ranges.

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

3. The detection steps in the assay must be carried out at 18°C to 28°C.

E. Time

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

F. Vortexing

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

G. Water Baths

1. The level of the water in the water baths must be maintained at 1.5 inches to 2.0 inches (3.8 cm to 5 cm) 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.

H. Decontamination

1. Surfaces and Pipettors

Laboratory bench surfaces and pipettors must be decontaminated regularly with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. 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 Aspiration Manifold

a. Place a new TTC into the TTC rack. Turn on the vacuum pump. Attach the aspiration manifold to the tips in the TTC. Aspirate all Wash Solution remaining in the priming trough of the Wash Solution dispense station. (Move the dispense manifold out of the way.)

b. Pour at least 100 mL of 0.5% to 0.7% (0.07 M to 0.1 M), or if preferred 2.5% to 3.5% (0.35 M to 0.5 M), sodium

hypochlorite solution into the priming trough. Aspirate all of the solution through the aspiration manifold.

c. Pour at least 100 mL of deionized water into the priming trough. Aspirate all of the water through the aspiration manifold.

d. Eject the tips into their original TTC.

e. Leave the vacuum pump on until the manifold tubing is dry to prevent back flow.

f. Decontaminate the aspiration manifold surfaces as described in *TCS Unit*.

3. TCS Waste Container

When the waste bottle is 25% full or weekly, remove the waste bottle from the Target Capture System.

a. Turn off the vacuum pump and allow the vacuum pressure to equalize.

b. Release the quick disconnect fittings between the waste bottle and overflow bottle, and the waste bottle and aspiration manifold.

c. Remove the waste bottle from the vacuum trap enclosure.

d. Remove the cap and carefully add 400 mL of 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution to the bottle (or 1 L if using a 10 L waste bottle).

Note: This may be done in a fume hood to avoid the release of fumes into the laboratory.

e. Cap the waste bottle and gently swirl the contents until fully mixed.

f. Let the waste bottle sit for 15 minutes and then dispose of the contents (waste).

g. Rinse the waste bottle with water to remove any remaining waste.

h. Cap the empty waste bottle and place it in the vacuum trap enclosure. Attach the quick disconnect fitting to the TCS unit. Carefully discard both gloves.

4. TCS Unit

Wipe the surfaces of the TCS unit, aspiration manifold, and surface of the wash buffer ejector tips with paper towels moistened with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Follow the bleach step with a water rinse and then dry the unit completely with paper towels.

5. Racks

Submerge the racks in 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution, ensuring that they are covered by the bleach solution. Keep the racks submerged for 10 minutes. Longer exposure could damage the racks. Rinse the racks thoroughly with water, place the racks on a clean absorbent pad, and allow the racks to air-dry thoroughly. To prolong the life of the racks, allow the racks to dry upright, not upside-down.

I. 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 in Deactivation Fluid 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. Powderless gloves are recommended.

J. Lab Contamination Monitoring Protocol

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

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

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

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

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

K. Troubleshooting

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

TIGRIS DTS System Test Procedure

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

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Reconstitution/Preparation of a New Kit

Reagent Reconstitution *should be performed prior to beginning any work on the TIGRIS DTS System.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents for the 250-test, 100-test, and 50-test kits, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
    a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors before attaching the reconstitution collar.
    b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
    c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 2, Step 1).
    d. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
    e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 2, Step 2).
    f. Slowly invert the assembled bottle and vial. Allow the solution to drain from the bottle into the vial (Figure 2, Step 3).
    g. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 2, Step 4).
    h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottle and vial again, tilting at a 45° angle to minimize foaming (Figure 2, Step 5). Allow all of the liquid to drain back into the bottle.
    i. Remove the reconstitution collar and vial (Figure 2, Step 6).
    j. Recap the bottle.
        - For 250- or 50-test bottles, peel and discard the top label. Record operator initials, the reconstitution date, and lyophilized reagent lot number on the remaining label (Figure 2, Step 7).
        - For 100-test bottles, record operator initials and the reconstitution date directly on the label.
    k. Discard the reconstitution collar and vial (Figure 2, Step 8).

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

Note: Thoroughly mix Amplification GC, Enzyme, Probe GC, and Selection Reagents by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents

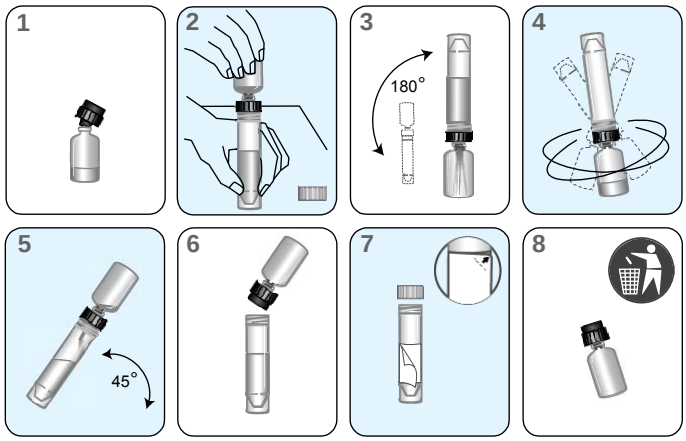

Figure 2. TIGRIS DTS Systems reconstitution process

2. To prepare working Target Capture Reagent (wTCR GC) for the 250-test or 100-test kit:
    a. Pair the appropriate bottles of TCR GC and TCR-B.
    b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
    c. Open the bottle of TCR GC, and set the cap on a clean, covered work surface.
    d. Open the bottle of TCR-B and pour the entire contents into the bottle of TCR GC. Expect a small amount of liquid to remain in the TCR-B bottle.
    e. Cap the bottle of TCR GC and gently swirl the solution to mix the contents. Avoid creating foam during this step.
    f. Record required information on the label.
        - For 250-test bottles, record operator initials and the current date on the label. Record the TCR-B lot number.
        - For 100-test bottles, record operator initials and the current date on the label.
    g. Discard the TCR-B bottle and cap.
3. To prepare working Target Capture Reagent (wTCR GC) for the 50-test kit:
    a. Pair the appropriate bottles of TCR GC and TCR-B.
    b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
    c. Open the bottle of TCR GC and set the cap on a clean, covered work surface.
    d. Open the bottle of TCR-B and set the cap on a clean, covered work surface.
    e. Remove the transfer pipette from its packaging.
    f. Transfer approximately 2 mL of TCR GC into the TCR-B bottle. **Do not** discard the transfer pipette at this step.
    g. Recap the TCR-B bottle and mix by inversion.
    h. Transfer the TCR GC plus TCR-B mixture from the TCR-B bottle back to the TCR GC bottle.
    i. Repeat Step h several times until the mixture in the TCR-B bottle is transferred back into the TCR GC bottle.
    j. Cap the bottle of TCR and gently swirl the solution to mix the contents. Ensure to avoid creating foam in this step.

k. Record operator initials and the current date on the label. Record the TCR-B lot number.

l. Discard the TCR-B bottle and cap. Discard the transfer pipette.

4. Prepare Selection Reagent

a. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.

b. Record operator initials and the current date on the label.

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

C. Reagent Preparation for Previously Reconstituted Reagents

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

2. If reconstituted Probe GC Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe GC Reagent may be used even if residual precipitate remains. Mix Probe GC Reagent by inversion, being careful not to induce foam, prior to loading onto the system.

3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.

4. Do not top off reagent bottles. The TIGRIS DTS System will recognize and reject bottles that have been topped off.

D. Specimen Handling

1. Allow the controls and specimens to reach room temperature prior to processing.

2. **Do not vortex specimens**.

3. Visually confirm that each specimen tube meets one of the following criteria:

a. The presence of a single blue APTIMA collection swab in a unisex swab specimen transport tube.

b. The presence of a single pink APTIMA collection swab in a vaginal swab specimen transport tube.

c. A final volume of urine between the black fill lines of a urine specimen transport tube.

d. The absence of a swab in the APTIMA specimen transport tube for PreservCyt Solution liquid Pap specimens.

4. Inspect specimen tubes before loading into rack:

a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.

b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.

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

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

Note: Failure to follow Steps 4a-c may result in liquid discharge from the transport tube cap.

Note: Up to three separate aliquots can be tested from each specimen. Attempts to pipette more than 3 aliquots from the specimen tube can lead to insufficient volume errors.

E. System Preparation

Set up the system and worklist according to instructions in the *TIGRIS DTS System Operator's Manual* and *Procedural Notes*.

Procedural Notes

A. Controls

1. To work properly with the TIGRIS APTIMA Assay software, front and end controls are required. The Positive Control, CT / Negative Control, GC must be in the first position and second to last position of a worklist. This control label is pink. The label text is "CONTROL + CT PCT / CONTROL – GC NGC". The Positive Control, GC / Negative Control, CT must be in the second position and last position of a worklist. This control label is blue-green. The label text is "CONTROL + GC PGC / CONTROL – CT NCT".

2. Each APTIMA control tube can be tested once. Attempts to pipette more than once from the tube can lead to insufficient volume errors.

B. Temperature

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

C. Glove Powder

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

D. Lab Contamination Monitoring Protocol for TIGRIS DTS System

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

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

1. Label swab transport tubes with numbers corresponding to the areas to be tested.

2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the swab transport medium, and swab the designated area using a circular motion.

3. Immediately insert the swab into a transport tube.

4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.

5. Recap the swab transport tube tightly.

6. Repeat Steps 2 to 5 for each area to be swabbed.

If the results are GC positive or equivocal, see *Test Interpretation - QC/Patient Results*. For additional TIGRIS DTS System-specific contamination monitoring information, see the *TIGRIS DTS System Operator's Manual*.

Test Interpretation - QC/Patient Results

A. Test Interpretation

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

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

*A zero (0 x 1000) RLU result on the run report represents a value between zero and 999 RLU. RLU values less than 160 on DTS Systems or 690 on TIGRIS DTS System will be reported as invalid.

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

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

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

B. Quality Control Results and Acceptability

The APTIMA Negative Control for GC, which is labeled "CONTROL + CT PCT / CONTROL – GC NGC," and the APTIMA Positive Control for GC, which is labeled "CONTROL + GC PGC / CONTROL – CT NCT," act as controls for the target capture, amplification, and detection steps of the assay. In accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations, additional controls for cell lysis and RNA stabilization may be included. The Positive Control for GC, which is labeled "CONTROL + GC PGC / CONTROL – CT NCT" contains non-infectious GC rRNA. If desired, additional controls can be ordered as a kit. See *Optional Materials*. Correct preparation of specimens is confirmed visually by the presence of a single APTIMA collection swab in a swab specimen transport tube, a final volume of urine in between the black fill lines of a urine specimen transport tube or the absence of a swab in the APTIMA Specimen Transfer tube for liquid Pap specimens.

The APTIMA Assay Controls must produce the following test results:

Control	Total RLU (x1000)	GC Result
Positive Control, CT / Negative Control, GC	0* and < 50	Negative
Positive Control, GC / Negative Control, CT	≥100 and < 12,000	Positive

*A zero (0 x 1000) RLU result on the run report represents a value between zero and 999 RLU. RLU values less than 160 on DTS Systems or 690 on TIGRIS DTS System will be reported as invalid.

1. The APTIMA Assay Software automatically evaluates the controls according to the above criteria and will report the Run Status as PASS if the run control criteria are met, and FAIL if the run control criteria are not met. If the Run Status is FAIL, all test results in the same run are invalid and must not be reported.
2. Each laboratory should implement appropriate control procedures to satisfy the requirements of CLIA regulations (section 493.1256).

Note: See *Lab Contamination Monitoring Protocol*, or contact Gen-Probe Technical Support for help with out-of-range controls on the DTS Systems.

3. 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.
4. Negative controls may not be effective in monitoring random carryover. See *TIGRIS DTS System Analytical Performance Characteristics* for results from a high-target analytical carryover study that was performed to demonstrate control of carryover on the TIGRIS DTS System.

C. Specimen Preparation Control (optional)

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

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, urine, and PreservCyt liquid Pap specimen results. See *Notes* below.

a. Initial results

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

b. Retest results

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

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

Notes:

- The first valid, non-equivocal result for each analyte is the result that should be reported.
- Careful consideration of performance data is recommended for interpreting APTIMA GC test results for asymptomatic individuals or any individuals in low prevalence populations.
- A negative result does not preclude the presence of a GC infection because results are dependent on adequate specimen collection,

absence of inhibitors, and sufficient rRNA to be detected. Test results may be affected by improper specimen collection, improper specimen storage, technical error, specimen mix-up, or target levels below the assay limit of detection.

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

Limitations

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

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

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

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

E. The APTIMA GC Assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications. For those patients for whom a false positive result may have adverse psycho-social impact, CDC recommends retesting by a method using an alternate technology (1).

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

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

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

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

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

K. For the vaginal swab, endocervical swab, male urethral swab and urine specimen clinical studies, performance characteristics for detecting GC are derived from high prevalence populations. Positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.

L. For the PreservCyt Solution liquid Pap specimen clinical studies, the APTIMA GC Assay performance for detecting GC is derived primarily from low prevalence populations. Nonetheless, positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.

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

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

O. The APTIMA GC Assay has not been validated for use with vaginal swab specimens collected by patients at home.

P. Performance of the vaginal swab specimen has not been evaluated in pregnant women.

Q. Performance of the vaginal swab and PreservCyt Solution liquid Pap specimen has not been evaluated in teenage women less than 16 years of age.

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

S. The performance of the TIGRIS DTS System has not been determined at altitudes above 7355 feet (2240 m). Additional volumetric verifications and assay specific studies will be performed prior to, or as part of, the installation and acceptance process in laboratories above 7355 foot (2240 m) altitude.

T. There is no evidence of degradation of nucleic acids in PreservCyt Solution. If a PreservCyt Solution liquid Pap specimen has small numbers of GC cellular material, uneven distribution of this cellular material may occur. Also, when compared to direct sampling with the APTIMA Swab Transport Media, the additional volume of PreservCyt Solution results in greater dilution of the sample material. These factors may affect the ability to detect small numbers of organisms in the collected material. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary.

U. Customers must independently validate an LIS transfer process.

Clinical Study Results

The performance characteristics of the APTIMA GC Assay were established in two clinical investigations conducted in North America. The first clinical investigation established the sensitivity, specificity, and predictive values of the APTIMA GC Assay using clinician-collected endocervical, vaginal, and male urethral swab specimens, patient-collected vaginal swab specimens, and male and female urine specimens. The first investigation also evaluated the precision of the APTIMA GC Assay when performed according to NCCLS Guidelines (12). The second clinical investigation established the sensitivity, specificity, and predictive values of the APTIMA GC Assay using PreservCyt transport medium (component of the ThinPrep 2000 System). PreservCyt Solution liquid Pap specimens were also evaluated for within-laboratory precision with the APTIMA GC Assay.

DTS Systems Expected Values

Prevalence

The prevalence of GC 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 GC in North America, by specimen type as determined by the APTIMA GC Assay is shown in Tables 1 and 1a for two clinical investigations. Refer to the *Clinical Specimen Study: Endocervical Swab, Male Urethral Swab, Vaginal Swab, and Urine Specimens* and *Clinical Specimen Study: PreservCyt Liquid Pap Specimens* sections in the *DTS Systems Clinical Performance Characteristics* section for a description of the clinical specimen performance characteristics.

Table 1: Prevalence of *N. gonorrhoeae* by Clinical Site and Overall as Determined by APTIMA GC Assay Results

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

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

Table 1a: Prevalence of *N. gonorrhoeae* by Clinical Site and Overall as Determined by APTIMA GC Assay Results Using PreservCyt Liquid Pap Solution Specimens

Site	% (#positive/#tested)	
1	5.0	(5/100)
2	0.8	(1/124)
3	0.8	(4/475)
4	1.4	(4/287)
5	0.0	(0/297)
6	0.5	(2/364)
All	1.0	(16/1647)

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

The estimated positive and negative predictive values (PPV and NPV) for different hypothetical prevalence rates using the APTIMA GC Assay are shown in Table 2. These calculations are based on hypothetical prevalence rates and the overall sensitivity and specificity estimated from the patient infected status. The overall sensitivity and specificity for GC was 97.6% and 99.3%, respectively (Table 2). The actual PPV and NPV for clinician-collected endocervical, vaginal and male urethral swab, patient-collect vaginal swab, and male and female urine specimens are shown in Table 6 for each clinical site and overall. The actual PPV and NPV for PreservCyt liquid Pap specimens are shown in Table 6a.

Table 2: Positive and Negative Predictive Values for Hypothetical Prevalence Rates in North America

Hypothetical Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1	97.6	99.3	58.7	100.0
2	97.6	99.3	74.1	100.0
5	97.6	99.3	88.1	99.9
10	97.6	99.3	94.0	99.7
15	97.6	99.3	96.1	99.6
20	97.6	99.3	97.2	99.4
25	97.6	99.3	97.9	99.2
30	97.6	99.3	98.4	99.0

APTIMA GC Assay RLU Distribution

Figure 3 shows the RLU distribution for the APTIMA GC Assay for the following specimen types tested in the clinical study: from symptomatic subjects, clinician-collected endocervical, vaginal, and male urethral swab specimens and patient-collected female and male urine specimens; and from asymptomatic subjects, clinician-collected endocervical and vaginal swab specimens and patient-collected vaginal swab, female and male urine specimens. Table 3 summarizes the RLU distribution for the total positive and total negative results, as well as the false positive and false negative results for these specimen types relative to infected patient status. Across certain specimen types, there is a trend toward an increasing proportion of true positives as the RLU values increase.

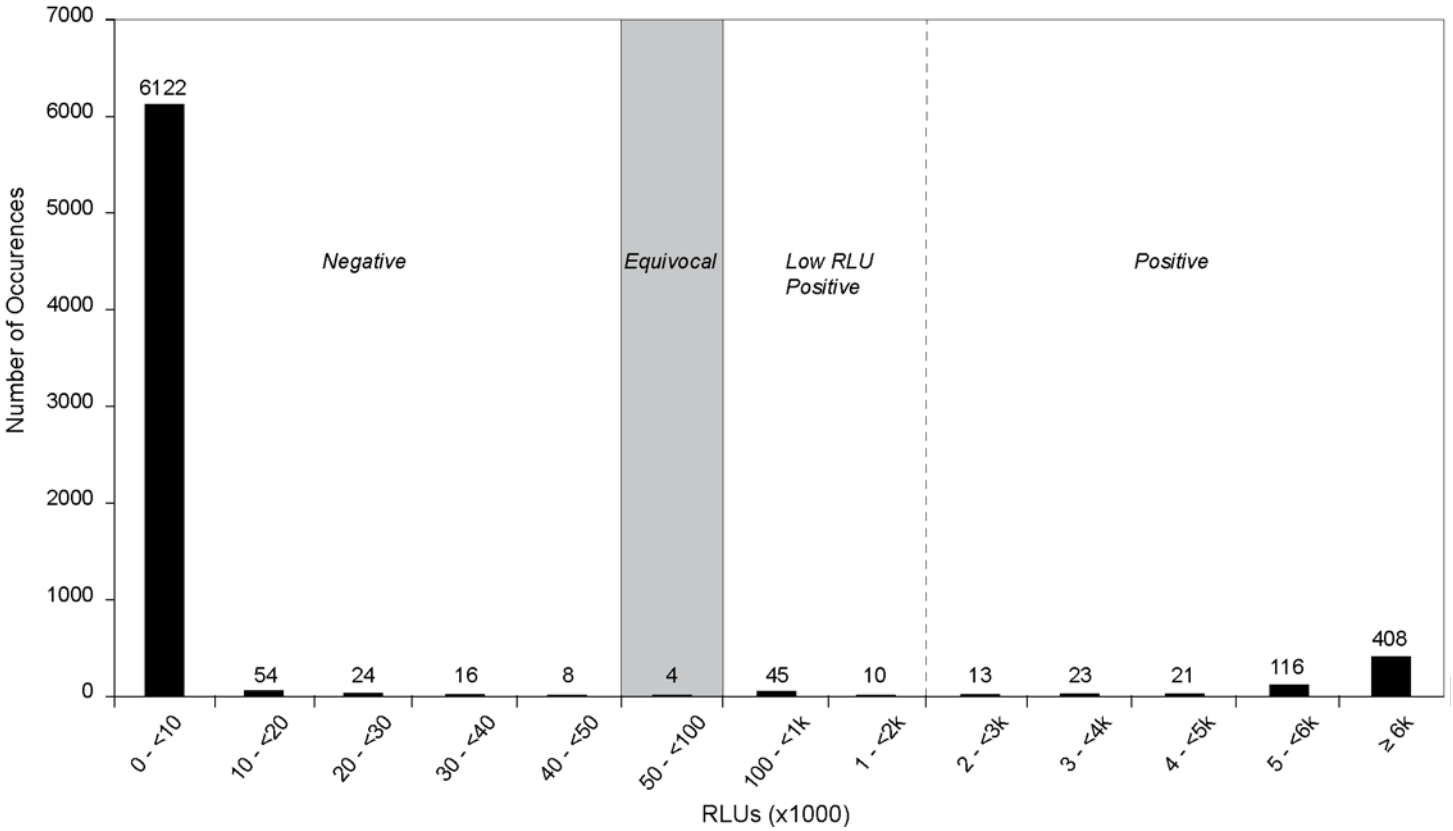

Figure 3. Frequency of RLU Distribution for the APTIMA GC Assay

Table 3: APTIMA GC Assay RLU Distribution

	RLUs (x 1000)											
	0 - <10	10 - <20	20 - <30	30 - <40	40 - <50	50 - <100	100 -<1K	1K - <2K	2K - <3K	3K - <4K	4K - <5K	5K - <6K	≥6K
Total Positives						-	45	10	13	23	21	116	408
Total False Positives						-	35	6	2	4	0	3	0
CVS						1	5	3	0	1	0	2	0
PVS						0	2	0	0	1	0	1	0
FS						2	12	1	0	0	0	0	0
MS						1	9	0	1	0	0	0	0
FU						0	2	0	0	1	0	0	0
MU						0	5	2	1	1	0	0	0
Total Negatives	**6122**	**54**	**24**	**16**	**8**	-							
Total False Negatives	**7**	**2**	**1**	**2**	**1**	-							
CVS	2	0	0	0	0	-							
PVS	0	0	0	0	0	-							
FS	0	0	0	1	1	-							
MS	0	1	0	0	0	-							
FU	3	1	1	1	0	-							
MU	2	0	0	0	0	-							

CVS = Clinician-Collected Vaginal Swab; **PVS** = Patient-Collected Vaginal Swab from asymptomatic subjects only; **FS** = Female Endocervical Swab; **MS** = Male Urethral Swab from symptomatic subjects only; **FU** = Female Urine; **MU** = Male Urine.
Shaded column denotes equivocal zone.

DTS Systems Clinical Performance Characteristics

See *TIGRIS DTS System Clinical Specimen Agreement* following the *DTS Systems Analytical Performance Characteristics* section for the TIGRIS DTS System-specific clinical performance characteristics.

Clinical Specimen Study: Endocervical Swab, Male Urethral Swab, Vaginal Swab, and Urine Specimens

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

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

All performance calculations were based on the total number of APTIMA GC Assay results for clinician-collected endocervical, vaginal and male urethral swab, and male and female urine specimens compared to a patient infected status algorithm for each gender. In the algorithm, the designation of a subject as being infected or not infected with GC was based on swab and urine specimen results from the commercially-available APTIMA COMBO 2 Assay and the other commercially-available NAAT. Subjects were considered infected with GC if two of the four swab and urine specimens tested positive in the APTIMA COMBO 2 Assay and the other reference NAAT (one specimen testing positive in each NAAT). Subjects were considered non-infected if less than two reference NAAT results were positive. Culture was not used as a reference test.

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

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

Table 4: Sensitivity and Specificity of the APTIMA GC Assay Relative to Patient Infected Status by Symptom Status and Overall for Male Urethral Swab, Male Urine, Female Endocervical Swab, Female Urine, Asymptomatic Patient-Collected Vaginal Swab and Clinician-Collected Vaginal Swab

Specimen		Symptom Status	N	TP	FP	TN	FN	Sensitivity (95% C.I.)		Specificity (95% C.I.)	
Male	Swab	Symptomatic	575	171	10[a]	393	1	99.4	(96.8 - 100)	97.5	(95.5 - 98.8)
	Urine	Symptomatic	576	171	4[b]	400	1	99.4	(96.8 - 100)	99.0	(97.5 - 99.7)
		Asymptomatic	745	9	5[c]	730	1	90.0	(55.5 - 99.7)	99.3	(98.4 - 99.8)
		All	1321	180	9[d]	1130	2	98.9	(96.1 - 99.9)	99.2	(98.5 - 99.6)
Female	Swab	Symptomatic	805	52	8[e]	744	1	98.1	(89.9 - 100)	98.9	(97.9 - 99.5)
		Asymptomatic	635	20	5[f]	609	1	95.2	(76.2 - 99.9)	99.2	(98.1 - 99.7)
		All	1440	72	13[g]	1353	2	97.3	(90.6 - 99.7)	99.0	(98.4 - 99.5)
	Urine	Symptomatic	810	48	2[h]	755	5	90.6	(79.3 - 96.9)	99.7	(99.0 - 100)
		Asymptomatic	639	21	1[i]	616	1	95.5	(77.2 - 99.9)	99.8	(99.1 - 100)
		All	1449	69	3[j]	1371	6	92.0	(83.4 - 97.0)	99.8	(99.4 - 100)
Patient-Collected	Vaginal Swab	Asymptomatic	629	21	4[k]	604	0	100	(83.9 - 100)	99.3	(98.3 - 99.8)
Clinician-Collected	Vaginal Swab	Symptomatic	809	52	7[m]	749	1	98.1	(89.9 - 100)	99.1	(98.1 - 99.6)
		Asymptomatic	637	21	4[n]	611	1	95.5	(77.2 - 99.9)	99.3	(98.3 - 99.8)
		All	1446	73	11[o]	1360	2	97.3	(90.7 - 99.7)	99.2	(98.6 - 99.6)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.
APTIMA COMBO 2 Assay GC results: # positive results / # specimens tested a: 2/10 b: 1/4 c: 1/5 d: 2/9 e: 5/8 f: 2/5 g: 7/13 h: 1/2 i: 1/1 j: 2/3 k: 3/4 l: 8/11 m: 6/7 n: 3/4 o: 9/11.

Clinical Specimen Study: PreservCyt Liquid Pap Specimens

A prospective multi-center clinical study was conducted to evaluate the use of the PreservCyt transport medium (a component of the ThinPrep 2000 System) as an alternative medium for gynecological specimens for the detection of *N. gonorrhoeae* by the APTIMA GC Assay. One thousand six hundred forty-seven (1,647) symptomatic and asymptomatic subjects attending OB/GYN, family planning, public health, women's, and STD clinics were enrolled and evaluated in the clinical study. Of these subjects, 1,288 were asymptomatic subjects and 359 were symptomatic subjects (Table 7e). Subjects were enrolled from sites with GC prevalence that ranged from 0.0% to 5.0% (Table 6a).

Two specimens were collected from each eligible subject: one PreservCyt liquid Pap specimen and one endocervical swab specimen. PreservCyt liquid Pap specimens were collected with the spatula/cyto-brush or a broom-like brush cervical sampling device. The distribution of cervical sampling devices is summarized in Table 5 by specimen collection site and overall.

PreservCyt liquid Pap specimens were processed in accordance with the ThinPrep 2000 Processor Operator's Manual and APTIMA Specimen Transfer Kit package insert. After processing the PreservCyt liquid Pap specimen with the ThinPrep 2000 Processor, the specimen was transferred into the APTIMA Specimen Transfer Kit for testing with the APTIMA GC Assay.

Sensitivity and specificity of the APTIMA GC Assay in PreservCyt liquid Pap specimens were calculated by comparing results to the patient infected status. The algorithm included APTIMA COMBO 2 Assay and APTIMA GC Assay results in endocervical swab specimens. Both reference NAATs were required to be positive to establish an infected patient status. At least one reference NAAT was required to be negative to establish a non-infected patient status. The one equivocal result that was obtained from a reference NAAT was considered to be discordant with the investigative assay for the purpose of calculating performance, and thus the patient infected status was categorized as non-infected (n=1). Table 7e summarizes the frequency of test outcomes for the endocervical swab specimens tested with the APTIMA COMBO 2 Assay and APTIMA GC Assay.

Table 5a shows the sensitivities and specificities of the APTIMA GC Assay by symptom status and overall. Overall sensitivity was 92.3% (12/13). In symptomatic and asymptomatic subjects, sensitivities were 100% (7/7) and 83.3% (5/6), respectively. Overall specificity was 99.8% (1630/1634). In symptomatic and asymptomatic subjects, specificities were 99.4% (350/352) and 99.8% (1280/1282), respectively.

Table 6a shows the sensitivities and specificities of the APTIMA GC Assay by specimen collection site and overall. Sensitivities ranged from 80.0% to 100%. Specificities ranged from 99.0% to 100%.

Table 5: Distribution of Cervical Sampling Device Used for PreservCyt Solution Liquid Pap Specimens

Cervical Sampling Device Used	Clinical Collection Site						Total
	1	2	3	4	5	6	
Spatula/Cytobrush	0	124	475	287	57	364	1307
Broom-Type Device	100	0	0	0	240	0	340

Table 5a: Sensitivity and Specificity of the APTIMA GC Assay Relative to Patient Infected Status by Symptom Status and Overall for PreservCyt Solution Liquid Pap Specimen

Symptom	APTIMA GC PreservCyt Solution Result	+/+	+/-	-/+	-/-	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Symptomatic	Positive	7	0	0	2	100 (7/7) (59.0 – 100)	99.4 (350/352) (98.0 – 99.9)
	Negative	0	0	0	350		
	Total	7	0	0	352		
Asymptomatic	Positive	5	0	1[1]	1	83.3 (5/6) (35.9 – 99.6)	99.8 (1280/1282) (99.4 –100)
	Negative	1	0	5	1275		
	Total	6	0	6	1276		
All	Positive	12	0	1	3	92.3 (12/13) (64.0 – 99.8)	99.8 (1630/1634) (99.4 – 99.9)
	Negative	1	0	5	1625		
	Total	13	0	6	1628		

+/+ = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay
+/- = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Negative endocervical swab specimen result in the APTIMA GC Assay
-/+ = Negative endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay
-/- = Negative endocervical swab specimen result in the APTIMA COMBO 2 Assay/Negative endocervical swab specimen result in the APTIMA GC Assay
[1]One specimen had a discordant result: Equivocal endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay.

Table 6: Sensitivity, Specificity, and Predictive Values of the APTIMA GC Assay Relative to Patient Infected Status by Clinical Site and Overall for Male Urethral Swab, Male Urine, Female Endocervical Swab, Female Urine, Asymptomatic Patient-Collected Vaginal Swab, and Clinician-Collected Vaginal Swab

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)		Specificity (95% C.I.)		PPV (%)	NPV (%)
Male	Swab	1	145	49	0	96	0	33.8	100	(92.7 - 100)	100	(96.2 - 100)	100	100
		2	177	66	8	102	1	37.9	98.5	(92.0 - 100)	92.7	(86.2 - 96.8)	89.2	99.0
		3	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
		4	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
		5	49	7	1	41	0	14.3	100	(59.0 - 100)	97.6	(87.4 - 99.9)	87.5	100
		6	150	37	1	112	0	24.7	100	(90.5 - 100)	99.1	(95.2 - 100)	97.4	100
		7	54	12	0	42	0	22.2	100	(73.5 - 100)	100	(91.6 - 100)	100	100
		8	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
		All	575	171	10	393	1	29.9	99.4	(96.8 - 100)	97.5	(95.5 - 98.8)	94.5	99.7
	Urine	1	252	53	1	198	0	21.0	100	(93.3 - 100)	99.5	(97.2 - 100)	98.1	100
		2	353	68	3	280	2	19.8	97.1	(90.1 - 99.7)	98.9	(96.9 - 99.8)	95.8	99.3
		3	4	0	0	4	0	0.0	N/A		100	(39.8 - 100)	N/A	100
		4	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
		5	200	8	3	189	0	4.0	100	(63.1 - 100)	98.4	(95.5 - 99.7)	72.7	100
		6	305	39	2	264	0	12.8	100	(91.0 - 100)	99.2	(97.3 - 99.9)	95.1	100
		7	207	12	0	195	0	5.8	100	(73.5 - 100)	100	(98.1 - 100)	100	100
		8	N/A	N/A	N/A	N/A	N/A	N/A	N/A		N/A		N/A	N/A
		All	1321	180	9	1130	2	13.8	98.9	(96.1 - 99.9)	99.2	(98.5 - 99.6)	95.2	99.8
Female	Swab	1	226	12	2	212	0	5.3	100	(73.5 - 100)	99.1	(96.7 - 99.9)	85.7	100
		2	197	29	3	164	1	15.2	96.7	(82.8 - 99.9)	98.2	(94.8 - 99.6)	90.6	99.4
		3	114	4	1	109	0	3.5	100	(39.8 - 100)	99.1	(95.0 - 100)	80.0	100
		4	260	5	1	254	0	1.9	100	(47.8 - 100)	99.6	(97.8 - 100)	83.3	100
		5	199	2	1	196	0	1.0	100	(15.8 - 100)	99.5	(97.2 - 100)	66.7	100
		6	294	19	5	269	1	6.8	95.0	(75.1 - 99.9)	98.2	(95.8 - 99.4)	79.2	99.6
		7	102	0	0	102	0	0.0	N/A		100	(96.4 - 100)	N/A	100
		8	48	1	0	47	0	2.1	100	(2.5 - 100)	100	(92.5 - 100)	100	100
		All	1440	72	13	1353	2	5.1	97.3	(90.6 - 99.7)	99.0	(98.4 - 99.5)	84.7	99.9
	Urine	1	227	11	2	213	1	5.3	91.7	(61.5 - 99.8)	99.1	(96.7 - 99.9)	84.6	99.5
		2	198	30	0	167	1	15.7	96.8	(83.3 - 99.9)	100	(97.8 - 100)	100	99.4
		3	113	4	0	109	0	3.5	100	(39.8 - 100)	100	(96.7 - 100)	100	100
		4	265	5	0	260	0	1.9	100	(47.8 - 100)	100	(98.6 - 100)	100	100
		5	199	2	0	197	0	1.0	100	(15.8 - 100)	100	(98.1 - 100)	100	100
		6	296	16	1	275	4	6.8	80.0	(56.3 - 94.3)	99.6	(98.0 - 100)	94.1	98.6
		7	102	0	0	102	0	0.0	N/A		100	(96.4 - 100)	N/A	100
		8	49	1	0	48	0	2.0	100	(2.5 - 100)	100	(92.6 - 100)	100	100
		All	1449	69	3	1371	6	5.2	92.0	(83.4 - 97.0)	99.8	(99.4 - 100)	95.8	99.6

Table 6: Sensitivity, Specificity, and Predictive Values of the APTIMA GC Assay Relative to Patient Infected Status by Clinical Site and Overall for Male Urethral Swab, Male Urine, Female Endocervical Swab, Female Urine, Asymptomatic Patient-Collected Vaginal Swab, and Clinician-Collected Vaginal Swab *(Continued)*

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)		Specificity (95% C.I.)		PPV (%)	NPV (%)
Patient-Collected	Vaginal Swab (Asymptomatic)	1	70	5	1	64	0	7.1	100	(47.8 - 100)	98.5	(91.7 - 100)	83.3	100
		2	46	7	1	38	0	15.2	100	(59.0 - 100)	97.4	(86.5 - 99.9)	87.5	100
		3	45	2	0	43	0	4.4	100	(15.8 - 100)	100	(91.8 - 100)	100	100
		4	152	1	0	151	0	0.7	100	(2.5 - 100)	100	(97.6 - 100)	100	100
		5	130	1	0	129	0	0.8	100	(2.5 - 100)	100	(97.2 - 100)	100	100
		6	75	5	2	68	0	6.7	100	(47.8 - 100)	97.1	(90.1 - 99.7)	71.4	100
		7	68	0	0	68	0	0.0	N/A		100	(94.7 - 100)	N/A	100
		8	43	0	0	43	0	0.0	N/A		100	(91.8 - 100)	N/A	100
		All	629	21	4	604	0	3.3	100	(83.9 - 100)	99.3	(98.3 - 99.8)	84.0	100
Clinician-Collected	Vaginal Swab	1	227	12	2	213	0	5.3	100	(73.5 - 100)	99.1	(96.7 - 99.9)	85.7	100
		2	197	30	3	163	1	15.7	96.8	(83.3 - 99.9)	98.2	(94.8 - 99.6)	90.9	99.4
		3	113	4	0	109	0	3.5	100	(39.8 - 100)	100	(96.7 - 100)	100	100
		4	263	5	3	255	0	1.9	100	(47.8 - 100)	98.8	(96.6 - 99.8)	62.5	100
		5	199	2	0	197	0	1.0	100	(15.8 - 100)	100	(98.1 - 100)	100	100
		6	295	19	3	272	1	6.8	95.0	(75.1 - 99.9)	98.9	(96.8 - 99.8)	86.4	99.6
		7	102	0	0	102	0	0.0	N/A		100	(96.4 - 100)	N/A	100
		8	50	1	0	49	0	2.0	100	(2.5 - 100)	100	(92.7 - 100)	100	100
		All	1446	73	11	1360	2	5.2	97.3	(90.7 - 99.7)	99.2	(98.6 - 99.6)	86.9	99.9

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

Table 6a: Sensitivity, Specificity and Predictive Values of the APTIMA GC Assay Relative to Patient Infected Status by Clinical Site and Overall for PreservCyt Solution Liquid Pap Specimens

Site	APTIMA GC PreservCyt Solution Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (%) (95% C.I.)	Specificity (%) (95% C.I.)	PPV(%)	NPV(%)
1	Positive	5	0	0	0	5.0	100 (5/5) (47.8 – 100)	100 (95/95) (96.2 – 100)	100	100
	Negative	0	0	0	95					
	Total	5	0	0	95					
2	Positive	1	0	0	0	0.8	100 (1/1) (2.5 – 100)	100 (123/123) (97.0 – 100)	100	100
	Negative	0	0	0	123					
	Total	1	0	0	123					
3	Positive	4	0	0	0	1.1	80.0 (4/5) (28.4 – 99.5)	100 (470/470) (99.2 – 100)	100	99.8
	Negative	1	0	0	470					
	Total	5	0	0	470					
4	Positive	1	0	0	3	0.3	100 (1/1) (2.5 – 100)	99.0 (283/286) (97.0 – 99.8)	25.0	100
	Negative	0	0	3	280					
	Total	1	0	3	283					
5	Positive	0	0	0	0	0.0	N/A	100 (297/297) (98.8 – 100)	N/A	100
	Negative	0	0	0	297					
	Total	0	0	0	297					
6	Positive	1	0	1[1]	0	0.3	100 (1/1) (2.5 – 100)	99.7 (362/363) (98.5 – 100)	50.0	100
	Negative	0	0	2	360					
	Total	1	0	3	360					
ALL	Positive	12	0	1	3	0.8	92.3 (12/13) (64.0 – 99.8)	99.8 (1630/1634) (99.4 – 99.9)	75.0	99.9
	Negative	1	0	5	1625					
	Total	13	0	6	1628					

N/A = not applicable
+/+ = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay
+/- = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Negative endocervical swab specimen result in the APTIMA GC Assay
-/+ = Negative endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay
-/- = Negative endocervical swab specimen result in the APTIMA COMBO 2 Assay/Negative endocervical swab specimen result in the APTIMA GC Assay
[1]One specimen had a discordant result: Equivocal endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay.

Table 7a: Symptomatic Male Urethral Swab Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

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

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing. **MS** = Symptomatic Male Urethral Swab; **MU** = Male Urine.

Table 7b: Male Urine Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

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

Sympt. = Symptomatic; **Asympt**. = Asymptomatic. **N/A** = Specimen not obtained or available for testing. The equal symbol (=) represents equivocal or indeterminate on repeat testing. **MS** = Male Urethral Swab; **MU** = Male Urine.

Table 7c: Female Endocervical Swab and Urine Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

Patient Infected Status	NAAT 1 (APTIMA COMBO 2 Assay)		NAAT 2		APTIMA GC Assay		Symptom Status		
	FS	FU	FS	FU	FS	FU	Sympt.	Asympt.	Total
Infected	+	+	+	+	+	+	43	16	59
Infected	+	+	+	+	+	-	2	0	2
Infected	+	+	+	-	+	+	2	1	3
Infected	+	+	+	-	+	-	0	1	1
Infected	+	+	+	N/A	+	+	1	0	1
Infected	+	+	-	+	+	+	1	1	2
Infected	+	+	-	-	+	+	1	1	2
Infected	+	-	+	+	+	-	1	0	1
Infected	+	-	+	-	+	+	0	1	1
Infected	+	-	+	-	+	-	2	0	2
Infected	-	+	+	+	-	+	1	0	1
Infected	-	+	-	+	-	+	0	1	1
Infected	-	+	-	+	=	+	0	1	1
Infected	-	-	+	+	-	-	1	0	1
Non-infected	+	-	-	-	+	-	4	1	5
Non-infected	+	-	-	-	-	-	1	0	1
Non-infected	-	+	-	-	-	-	1	0	1
Non-infected	-	-	+	-	+	-	1	0	1
Non-infected	-	-	+	-	-	-	5	2	7
Non-infected	-	-	-	+	-	-	2	2	4
Non-infected	-	-	-	-	+	-	1	2	3
Non-infected	-	-	-	-	-	+	1	0	1
Non-infected	-	-	-	-	-	-	718	589	1307
Non-infected	-	-	-	-	=	-	1	0	1
Non-infected	-	-	-	N/A	-	-	2	3	5
Non-infected	-	-	-	=	-	-	11	11	22
Non-infected	-	-	=	-	-	-	1	1	2
Non-infected	-	N/A	-	-	-	N/A	1	1	2
Non-infected	N/A	-	-	-	N/A	-	5	4	9
Non-infected	=	-	-	-	+	-	1	1	2
Total							811	640	1451

Sympt. = Symptomatic; **Asympt**. = Asymptomatic. **N/A** = Specimen not obtained or available for testing. The equal symbol (=) represents equivocal or indeterminate on repeat testing. **FS** = Female Endocervical Swab; **FU** = Female Urine.

Table 7d: Vaginal Swab Results from Subjects Infected or Non-Infected with *N. gonorrhoeae* According to Patient Infected Status

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

Sympt. = Symptomatic; Asympt. = Asymptomatic. N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing. **FS** = Female Endocervical Swab; **FU** = Female Urine; **PVS** = Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab.

Table 7e: PreservCyt Liquid Pap Specimen Clinical Study Patient Infected Status Results for *N. gonorrhoeae*

Patient Infected Status	Endocervical Swab		Symptom Status	
	APTIMA COMBO 2 Assay	APTIMA GC Assay	Symptomatic	Asymptomatic
Infected	Positive	Positive	7	6
Non-Infected	Negative	Negative	352	1276
Non-Infected	Negative	Positive	0	5
Non-Infected	Equivocal	Positive	0	1
Total			359	1288

RLU Distribution of APTIMA Controls

The distribution of the RLUs for the APTIMA Positive Control, GC / Negative Control, CT and the APTIMA Positive Control, CT / Negative Control, GC from all the APTIMA GC Assay runs performed during the clinical specimen study is presented in Table 8.

Table 8: Distribution of RLU of the APTIMA Controls During the Clinical Specimen Studies Including Endocervical, Vaginal and Male Urethral Swab, Male and Female Urine Specimens, and PreservCyt Liquid Pap Studies

Control	Statistics	RLU (x1000)	
		Swab and Urine Specimen Clinical Study	PreservCyt Liquid Pap Specimen Clinical Study
Positive Control, GC / Negative Control, CT	N	193	218
	Mean	5048	4561
	SD	1071	1295
	Maximum	6765	6791
	75th Percentile	5763	5450
	Median	5175	4859
	25th Percentile	4645	3804
	Minimum	229	158
Positive Control, CT / Negative Control, GC	N	193	218
	Mean	2.15	2.60
	SD	2.20	2.80
	Maximum	20	29
	75th Percentile	2	3
	Median	2	2
	25th Percentile	1	2
	Minimum	0	1

Precision Study

APTIMA GC Assay precision (i.e., reproducibility) was evaluated at two external clinical sites and at Gen-Probe. APTIMA GC Assay precision was evaluated across three APTIMA GC Assay kit lots, three study sites, six operators and 108 APTIMA GC Assay runs. Two operators at each of the three testing sites performed a total of six APTIMA GC Assay runs per kit lot for a total of 36 runs per kit lot. Each run was composed of a 12-member precision panel containing 0 to 2,433 fg/assay of GC rRNA. Reproducibility was established using swab transport medium spiked with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Table 9 presents the precision RLU data in terms of Mean, Standard Deviation, Coefficient of Variation (CV), and percent agreement with expected results for calculations of between-site, between-operator, between-lot, between-run, and within-run variability.

Table 9: APTIMA GC Assay Precision Data Using a 12-Member Precision Panel Containing 0 to 2,433 fg/assay of GC rRNA

Concentration	N	Mean RLU (x1000)	% Agrmt.	Within-Run		Between-Site		Between-Lot		Between-Operator		Between-Run	
				SD RLU (x1000)	CV (%)	SD RLU (x1000)	CV (%)	SD RLU (x1000)	CV (%)	SD RLU (x1000)	CV (%)	SD RLU (x1000)	CV (%)
Neg (0 fg/mL)	540	11.7	99.8	233.3	N/A	0	N/A	0	N/A	4.3	N/A	0	N/A
Low (608-625 fg/mL)	324	5574.4	99.7	617.2	11.1	189.2	3.4	518.1	9.3	311.3	5.6	527.4	9.5
Mid (6,082 fg/mL)	108	6502.6	100	138.8	2.1	0	0.0	481.9	7.4	514.8	7.9	579.4	8.9
High (12,500 fg/mL)	324	6786.0	100	270.3	4.0	0	0.0	581.3	8.6	410.7	6.1	647.1	9.5

SD = Standard Deviation; **CV(%)** = Percent Coefficient of Variation;**% Agrmt**. = Percent Agreement. **N/A** = not applicable for negative analyte.
Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and %CV is set to zero (12).

PreservCyt liquid Pap specimen within-laboratory precision with the APTIMA GC Assay was determined by spiking PreservCyt vials with 20 GC CFU per vial (0.1 CFU per reaction) and 100 GC CFU per vial (0.5 CFU per reaction). Vials containing 10,000 GC CFU per vial (50 CFU per reaction) and unspiked PreservCyt vials were tested as positive and negative controls. Ten vials spiked at each CFU level and ten unspiked vials were divided between two operators. The operators vortexed the vials and then transferred 14 aliquots (1.0 mL each) per vial into 14 APTIMA Transfer Tubes as per the APTIMA Specimen Transfer Kit package insert. The operators were blinded to the samples' titers. Each of the resulting Pap-STM samples was tested once in the APTIMA GC Assay. A total of five runs were performed over a five day period for 140 results at the 0.1, 0.5, and 50 CFU level. There were 136 valid results and 4 invalid results for the negative control panel. The invalid results were due to a misplacement of a TTU in the Leader HC+. The results are summarized in Table 10.

Table 10: APTIMA GC Assay Within-Laboratory Precision Data for PreservCyt Using a 4-Member Precision Panel Containing 0 to 500 CFU/mL GC Cells

Panel Member	CFU/mL PreservCyt	CFU/rxn	n	Agreed	% Agrmt.	Mean RLU (x1000)	Within-Operator		Between-Day		Between-Operator		Total	
							SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
A	1	0.1	140	39	27.9	313.7	758.3	241.7	132.5	42.2	0.0	0.0	769.8	245.4
B	5	0.5	140	113	80.7	1211.1	1031.3	85.2	169.8	14.0	150.4	12.4	1056.0	87.2
C	500	50	140	140	100	5636.8	220.7	3.9	135.7	2.4	0.0	0.0	259.1	4.6
D	0	0	136*	136	100	1.2	0.5	N/A	0	N/A	0.3	N/A	0.6	N/A

* There were four invalid results due to a misplaced TTU in the Leader HC+.
Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is small. When this occurs, the variability as measured with SD and %CV is set to zero (12). N/A = Not applicable for negative panel members. Operator = Run. Samples with discordant results were included in the signal variability analysis.

DTS Systems Analytical Performance Characteristics

See *TIGRIS DTS System Analytical Performance Characteristics* following the *TIGRIS DTS System Clinical Specimen Agreement* section for the TIGRIS DTS System-specific analytical performance characteristics.

Analytical Sensitivity

N. gonorrhoeae analytical sensitivity (limit of detection) was determined by directly comparing dilutions of 51 different clinical isolates in culture and in the APTIMA GC Assay. The analytical sensitivity claim for the assay is 50 CFU/assay (362 CFU/swab, 250 CFU/mL urine, and 487.5 CFU/mL PreservCyt Solution liquid Pap).

Analytical Specificity

A total of 154 culture isolates were evaluated using the APTIMA GC Assay. These isolates included 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, parasites and viruses. All organisms except *C. psittaci, C. pneumoniae, U. urealyticum* and the viruses were tested at 1.0×10^6 cells/assay in KOVA-Trol urine transport media and 60 organisms were tested in Swab Transport Media. The Chlamydia and Neisseria organisms were tested in the PreservCyt Solution media. *C. psittaci* VR601 was tested at 8.0×10^4 cells/assay and *C. psittaci* VR125 was tested at 1.0×10^5 cells/assay. *C. pneumoniae* was tested at 4.0×10^3 cells/assay and *U. urealyticum* was tested at 6.7×10^6 cells/assay. The viruses were tested as follows: (a) herpes simplex virus I: 2.5×10^4 $TCID_{50}$/assay, (b) herpes simplex virus II: 6.0×10^4 $TCID_{50}$/assay, (c) human papillomavirus 16: 2.9×10^6 DNA copies/assay and (d) cytomegalovirus: 4.8×10^5 cells/assay. The list of organisms tested is shown in Table 11.

Table 11: Analytical Specificity

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

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

Interfering Substances

The following interfering substances were individually spiked into swab, PreservCyt liquid Pap, and/or urine specimens: 10% blood, contraceptive jelly, spermicide, moisturizer, hemorrhoidal anesthetic, body oil, powder, anti-fungal cream, vaginal lubricants, feminine spray and leukocytes (1.0×10^6 cells/mL). The following interfering substances were individually spiked into urine specimens: 30% blood, urine analytes, protein, glucose, ketones, bilirubin, nitrate, urobilinogen, pH 4 (acidic), pH 9 (alkaline), leukocytes (1.0×10^6 cells/mL), cellular debris, vitamins, minerals, acetaminophen, aspirin and ibuprofen. All were tested for potential assay interference in the absence and presence of GC at the estimated rRNA equivalent of 50 GC cells/assay (250 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism.

No interference was observed with any of the tested substances. No inhibitors of amplification were observed in the APTIMA GC Assay.

Recovery

Escherichia coli, Gardnerella vaginalis, *Lactobacillus acidophilus*, *Bacteroides ureolyticus,* and *Staphylococcus epidermidis* (1.0×10^8 cells/assay) were added to samples containing the rRNA equivalent of approximately 50 GC cells (250 fg). These additions did not interfere with the amplification and detection of GC rRNA using the APTIMA GC Assay.

Specimen Stability Studies

A. Swab and Urine Specimens

Data to support the recommended shipping and storage conditions for endocervical, urethral and vaginal swab samples were generated with pooled negative swab samples. Pooled samples were spiked with GC at a final concentration of approximately 50 CFU per reaction. The spiked samples were held at -70°C, -20°C, 4°C, and 30°C. Samples were tested in duplicate at days 0, 20, 77, and 117. All test conditions were positive for GC at all times and temperatures.

Data to support the recommended shipping and storage conditions for urine samples were generated with female and male negative urine samples. The urine samples were spiked with GC at a final concentration of 100 CFU per reaction. The samples were held at 30°C for 24 hours prior to being added to the urine transport media (UTM). The UTM samples then were held at 4°C and 30°C and tested in triplicate at days 1,14, 32 and 35. The UTM samples were also stored at -20°C and -70°C and tested in triplicate at days 1, 35, and 109. All replicates were positive for GC with UTM samples held at 4°C and -70°C. When the UTM samples were held at 30°C, 94% of the replicates were positive for GC at day 35. When the UTM samples were held at -20°C, 98% of the replicates were positive for GC at day 109.

B. PreservCyt Solution Liquid Pap Specimens

Data to support the recommended shipping and storage conditions for PreservCyt Solution liquid Pap samples were generated with negative processed and unprocessed liquid Pap samples. For the unprocessed samples, four pools of PreservCyt Solution samples were tested after being stored in the Cytyc PreservCyt Solution vial. Each specimen pool was spiked with 50-100 CFU GC/assay, held at 2°C, 10°C, and 30°C, then tested at baseline and on days 5, 7, 8, 14, 18, 21, 25 and 36. All of the spiked samples were positive for GC at all times and temperatures.

For the processed samples, four pools of PreservCyt Solution samples were used to determine processed specimen stability at 2°C to 30°C. Each negative sample pool was spiked with 50-100 CFU GC/assay, then tested at baseline. Prior to processing, the PreservCyt Solution samples were stored at 30°C for seven (7) days to simulate the time lapse between sample collection, Pap processing and shipment to a microbiology testing lab. After seven days at 30°C, 1 mL aliquots of each pool were transferred to an APTIMA Specimen Transfer Tube and tested at baseline before being placed at 2°C, 10°C, and 30°C. The processed samples were then tested for 17 days stored at 30°C and 36 days stored at 2°C to 10°C. All of the spiked samples were positive for GC at all times and temperatures.

Data to support longer storage conditions were generated from four pools of negative processed PreservCyt Solution samples tested at below freezing temperatures. Each pool was spiked with 50-100 CFU GC/assay, then tested at baseline. Each pool was first placed at 30°C for 14 days and then stored at -20°C or -70°C over the course of 106 days. All of the spiked samples were positive for GC at all times and temperatures.

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

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

TIGRIS DTS System Clinical Specimen Agreement

TIGRIS DTS System Agreement

Agreement between APTIMA GC Assay results generated on the fully automated TIGRIS DTS System and semi-automated DTS Systems was evaluated by testing endocervical swab, male urethral swab, male and female urine, vaginal swab, and PreservCyt liquid Pap specimens. Each of the clinical specimens was tested individually with the APTIMA GC Assay on both the TIGRIS DTS System and DTS Systems at Gen-Probe. The order of testing was not randomized. Specimens identified for inclusion were tested on the TIGRIS DTS System followed by testing on DTS Systems.

Clinical Specimen Agreement Study - Endocervical Swab, Male Urethral Swab, Female and Male Urine, Vaginal Swab, and PreservCyt Liquid Pap Specimens

Female and male subjects attending STD, family planning, and OB/GYN clinics from eight geographically diverse sites with low to high prevalence for GC contributed endocervical swab, male urethral swab, male and female urine, vaginal swab, and PreservCyt liquid Pap specimens. The specimens were transferred directly to Gen-Probe for testing. At Gen-Probe, endocervical swab, male urethral swab, male and female urine specimens were first screened with APTIMA COMBO 2 Assay on the TIGRIS DTS System. The vaginal swab and PreservCyt liquid Pap specimens were screened with the APTIMA COMBO 2 Assay on the DTS Systems. Specimens with final invalid or equivocal results were not selected in the APTIMA GC Clinical Specimen Agreement Study.

One hundred twenty-nine female swabs (70 endocervical and 59 vaginal), 133 male urethral swab, 72 female urine, 130 male urine, and 51 PreservCyt liquid Pap specimens with APTIMA COMBO 2 Assay GC positive and negative results were selected for comparison testing between the TIGRIS DTS System and the DTS Systems for the APTIMA GC Assay. The majority of specimens (88 female swabs, 93 male swab, 47 female urine, 70 male urine, and 34 PreservCyt liquid Pap specimens) included for comparison testing were from symptomatic individuals. Specimens with initial invalid or equivocal results were retested using the same system on which the result was generated. Three female urine, 1 vaginal swab, and 1 male urethral swab specimens had initial equivocal results on the DTS Systems, upon retest, all had valid results. One male and 1 female urine specimen had initial invalid results on the TIGRIS DTS System, upon retest, both results were valid.

Table 12 shows the positive, negative, and overall agreements for all paired results for each specimen type by symptomatic status. Female swab specimens (endocervical and vaginal swabs combined), are imbalanced relative to positive and negative samples from symptomatic subjects, but overall agreement for symptomatic subjects was 100%, for asymptomatic subjects was 97.6% (40/41), and for ‘all’ (symptomatic and asymptomatic combined) overall agreement was 99.2% (128/129). For male urethral swab specimens, overall agreement for symptomatic, asymptomatic, and ‘all’ subjects was 100%. For female urine specimens, overall agreement for symptomatic subjects was 100%, for asymptomatic subjects was 96.0% (24/25), and 'all' was 98.6% (71/72).

For male urine specimens, overall agreement for symptomatic subjects was 98.6% (69/70), for asymptomatic subjects was 100%, and 'all' was 99.2% (129/130). For PreservCyt liquid Pap specimens, overall agreement for symptomatic, asymptomatic, and 'all' subjects was 100%. Because of the

relatively smaller specimen number from asymptomatic subjects, these findings may not be generalizable to APTIMA GC TIGRIS DTS System testing with specimens from asymptomatic subjects.

Refer to Table 4 for APTIMA GC Assay performance estimates for endocervical swab, vaginal swab, male urethral swab, and male and female urine specimens and to Table 5a for PreservCyt liquid Pap specimens tested on the DTS Systems. Clinical performance estimates for the TIGRIS DTS System with endocervical swab, vaginal swab, male urethral swab, male and female urine, and PreservCyt liquid Pap specimens would be expected to be similar given the agreement findings.

Table 12: Clinical Specimen Agreement Study: Positive, Negative, and Overall Agreements by Symptom Status

Symptom	Specimen	Gender	n	DTS+ TIGRIS+	DTS+ TIGRIS-	DTS- TIGRIS+	DTS- TIGRIS-	Positive % Agreement (95% CI)	Negative % Agreement (95% CI)	Overall % Agreement (95% CI)
Sympt.	Swab	Female*	88	55	0	0	33	100 (93.5-100)	100 (89.4-100)	100 (95.9-100)
		Male	93	66	0	0	27	100 (94.6-100)	100 (87.2-100)	100 (96.1-100)
	Urine	Female	47	24	0	0	23	100 (85.8-100)	100 (85.2-100)	100 (92.5-100)
		Male	70	60	1	0	9	98.4 (91.2-100)	100 (66.4-100)	98.6 (92.3-100)
	PreservCyt	Female	34	28	0	0	6	100 (87.7-100)	100 (54.1-100)	100 (89.7-100)
Asympt.	Swab	Female*	41	23	0	1[1]	17	100 (85.2-100)	94.4 (72.7-99.9)	97.6 (87.1-99.9)
		Male	40	7	0	0	33	100 (59.0-100)	100 (89.4-100)	100 (91.2-100)
	Urine	Female	25	9	0	1	15	100 (66.4-100)	93.8 (69.8-99.8)	96.0 (79.6-99.9)
		Male	60	5	0	0	55	100 (47.8-100)	100 (93.5-100)	100 (94.0-100)
	PreservCyt	Female	17	12	0	0	5	100 (73.5-100)	100 (47.8-100)	100 (80.5-100)
All	Swab	Female*	129	78	0	1[1]	50	100 (95.4-100)	98.0 (89.6-100)	99.2 (95.8-100)
		Male	133	73	0	0	60	100 (95.1-100)	100 (94.0-100)	100 (97.3-100)
	Urine	Female	72	33	0	1	38	100 (89.4-100)	97.4 (86.5-99.9)	98.6 (92.5-100)
		Male	130	65	1	0	64	98.5 (91.8-100)	100 (94.4-100)	99.2 (95.8-100)
	PreservCyt	Female	51	40	0	0	11	100 (91.2-100)	100 (71.5-100)	100 (93.0-100)

"+" denotes a positive result, "-" a negative result, CI = confidence interval
*Endocervical and Vaginal Swab samples combined
[1]One disagreement in Vaginal Swab

Precision Study

The effect of several factors on the variability of APTIMA GC Assay performance on the TIGRIS DTS System was evaluated using 12-member STD reproducibility panels. Panel members contained 0 to 250,000 fg GC rRNA/assay. The panel included panel members with GC concentrations at the analytical sensitivity claim of 250 fg GC rRNA/assay.

The panels were tested at 1 external testing site and at Gen-Probe using 2 APTIMA GC Assay reagent lots. At Gen-Probe, 2 operators each performed 3 valid worklists per reagent lot on each of 2 TIGRIS DTS System instruments. At the external testing site, 2 operators each performed 3 valid worklists per reagent lot on 1 TIGRIS DTS System instrument. One worklist consisted of run controls and six 12-member panels. Samples with initial invalid or equivocal results from valid assay worklists were not retested. Eleven samples had final invalid results and were excluded from the reproducibility analyses.

Reproducibility was determined by calculating the agreement between the final assay results and the expected outcome for each panel member. Reproducibility was also assessed by calculating the SD and coefficient of variation (CV) of signal with respect to sites, operators, lots, and worklists. CVs were not calculated for GC-negative panel members due to low signal values that could theoretically equal zero. Table 13 shows the reproducibility results. All APTIMA GC Assay results on the TIGRIS DTS System agreed with the expected results for panel members containing 0, 250, 25,000, and 250,000 fg GC rRNA/assay. For panel members containing 2,500 fg GC rRNA/assay, agreement with expected results was 99.8%. CV values were less than or equal to 9.0%. These data indicate good reproducibility of the APTIMA GC Assay using the TIGRIS DTS System.

Table 13: TIGRIS DTS System Precision Data

Conc (fg rRNA per assay)	n	Mean RLU (x1000)	% Agrmt	Between-Site		Between-Operator		Between-Lot		Between-Worklist		Within-Worklist	
				SD (x1000)	CV (%)	SD[1] (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
0	859[2]	4.6	100	1.7	N/A	0.0	N/A	0.3	N/A	0.7	N/A	2.7	N/A
250	429[3]	4148	100	236	5.7	170	4.1	212	5.1	94.9	2.3	222	5.3
2,500	429[4]	5361	99.8	275	5.1	145	2.7	273	5.1	25.1	0.5	482	9.0
25,000	430[5]	5871	100	325	5.5	163	2.8	303	5.2	106	1.8	176	3.0
250,000	431[6]	6037	100	317	5.2	167	2.8	303	5.0	126	2.1	186	3.1

Agrmt = Agreement, Conc = Concentration, CV = Coefficient of variation, N/A = Not applicable for negative samples, RLU = Relative Light Units, SD = Standard deviation

[1] SD and CV values are set to 0 and 0.0%, respectively, according to the random effects model, if the variability due to this source relative to random errors and/or variation of other sources is numerically negative.

[2] There were 4 samples excluded from this analysis due to final invalid results. Additionally, one worklist was missing 1 replicate each of a GC-negative panel member.

[3] There were 3 samples excluded from this analysis due to final invalid results.

[4] There were 2 samples excluded from this analysis due to final invalid results. Additionally, two worklists were missing 1 replicate each of a panel member with 2,500 fg GC rRNA/assay and one worklist included 1 additional replicate of a panel member with 2,500 fg GC rRNA/assay.

[5] There were 2 samples excluded from this analysis due to final invalid results. Additionally, one worklist included 1 additional replicate of a panel member with 25,000 fg GC rRNA/assay. The same worklist was missing 1 replicate of another panel member with 25,000 fg GC rRNA/assay.

[6] One worklist was missing 1 replicate of a panel member with 250,000 fg GC rRNA/assay.

Note: Samples with invalid test results were excluded. Signal variability analysis includes samples with discordant results.

TIGRIS DTS System Analytical Performance Characteristics

Analytical Sensitivity Equivalence Study

Sensitivity panels in endocervical swab pool, vaginal specimen pool, urine specimen pool, and PreservCyt liquid Pap specimen pool were prepared at GC 250 fg/Assay rRNA and tested 60 replicates on the TIGRIS DTS System. Percent positivity (95% C.I.) on the TIGRIS DTS System for endocervical swab specimen was 100% (95.1 - 100), for vaginal swab specimen was 100% (95.1 - 100), for urine specimen was 100% (95.1 - 100), and PreservCyt liquid Pap specimen was 100% (95.1 - 100).

GC rRNA Spiked Clinical Panel Study

The GC rRNA spiked clinical panel study evaluated agreement between the two systems using six Gen-Probe prepared GC clinical panels spiked with 0 to 250,000 fg rRNA/assay of GC. The GC clinical panels were created from endocervical swab, vaginal swab, urethral swab, male urine, female urine, and PreservCyt liquid Pap specimens that had negative APTIMA GC results on the DTS Systems when tested at Gen-Probe. The negative specimens were pooled by specimen type, spiked or not spiked with GC rRNA and aliquotted as replicates of each panel member. Replicates of each of 6-panel members with different spiked rRNA levels were combined to create one clinical panel for each specimen type. Each panel contained a total of 132 replicates.

The initial male and female urine data show that some panel members that contained rRNA at a level below the claimed analytical sensitivity yielded unexpected negative results on the TIGRIS DTS System. Two follow-up studies were conducted to demonstrate and confirm agreement to expected results in spiked male or female urine panels. The original study design combined negative samples into a single master pool. The follow-up study design for male and female urine specimens was amended. The specimens were aliquotted into confirmed negative mini-pools to make the positive and negative panels. One hundred thirty-eight replicates were created for each panel.

Table 14 shows the percent agreement for each level of rRNA in the endocervical swab, vaginal swab, urethral swab, male urine, female urine, and PreservCyt liquid Pap panels, respectively, with expected GC results for the TIGRIS DTS System and for the DTS Systems. The concentration ranged from 1 log below to 3 logs above the 250 fg rRNA/assay for GC. Also shown in Table 14 are the overall percent agreements of the clinical panel study between the TIGRIS DTS System and DTS Systems.

Table 14: GC rRNA Spiked Clinical Panel Agreement Study

Specimen		Panel Member	Concentration (fg rRNA/Assay)	Replicates	TIGRIS % Agreement	DTS % Agreement	Overall % Agreement between TIGRIS and DTS (95% CI)
Swab	Endocervical	No Target	0	12	100	100	100 (97.2-100)
		Very Low	25	30	100	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
	Vaginal	No Target	0	12	100	100	100 (97.2-100)
		Very Low	25	29*	100	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
	Urethral	No Target	0	12	100	100	100 (97.2-100)
		Very Low	25	30	100	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
Male Urine	Initial Study	No Target	0	12	100	100	91.7 (85.6-95.8)
		Very Low	25	30	63.3 (19/30)	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
	Follow-up 1	No Target	0	18	100	100	100 (97.4-100)
		Very Low	25	30	100	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
	Follow-up 2	No Target	0	18	100	100	100 (97.4-100)
		Very Low	25	30	100	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
Female Urine	Initial Study	No Target	0	12	100	100	75.8 (67.5-82.8)
		Very Low	25	30	13.3 (4/30)	100	
		Low	250	30	80 (24/30)	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
	Follow-up 1	No Target	0	18	100	100	99.3 (96.0-100)
		Very Low	25	30	96.7 (29/30)	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	
	Follow-up 2	No Target	0	18	100	100	97.8 (93.8-99.5)
		Very Low	25	30	90 (27/30)	100	
		Low	250	30	100	100	
		Medium	2,500	30	100	100	
		High	250,000	30	100	100	

*Not tested on both systems due to insufficient sample volume

Table 14: GC rRNA Spiked Clinical Panel Agreement Study *(Continued)*

Specimen	Panel Member	Concentration (fg rRNA/Assay)	Replicates	TIGRIS % Agreement	DTS % Agreement	Overall % Agreement between TIGRIS and DTS (95% CI)
PreservCyt liquid Pap	No Target	0	12	100	100	100 (97.2-100)
	Very Low	25	30	100	100	
	Low	250	30	100	100	
	Medium	2,500	30	100	100	
	High	250,000	30	100	100	

*Not tested on both systems due to insufficient sample volume

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 GC Assay are identical between the TIGRIS DTS System and the DTS Systems, analytical specificity experiments on the TIGRIS DTS System were designed to focus on the most challenging culture isolates. These organisms included those known to cross-react in other amplification assays. Twenty-four (24) culture isolates were selected from the panel of organisms in Table 11, including 17 organisms that are most closely related to GC. All of the organisms tested produced negative results with the exception of one (1/648) false positive result. This was observed with *C. pneumoniae* where 1 replicate of 27 tested gave a false result. Repeat testing did not support cross-reactivity with this organism *(C. pneumoniae)*, as no positive tests were observed with 6 additional test replicates.

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 Systems. Fresh blood was added to clinical swab, vaginal swab, urine, and PreservCyt liquid Pap specimen pools, then tested for potential assay interference in the absence and presence of GC target at the estimated rRNA equivalent of 50 GC CFU/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, vaginal swab specimens, PreservCyt liquid Pap specimens, and 30% blood in urine specimens. All samples that did not contain target were negative for GC. These results indicate that, at the levels tested, whole blood is unlikely to affect the GC result on the TIGRIS DTS System.

Carryover Studies

To establish that the TIGRIS DTS System minimizes the risk of false positive results arising from carryover contamination, a study was conducted using spiked panels on three TIGRIS DTS Systems. The study used 20% high-target GC samples containing 1.0×10^9 cells/reaction, which were randomly spaced amongst 80% negative samples containing swab transport media. In the study, 576 high-target samples and 2,376 negative samples were tested across the three TIGRIS DTS Systems. Table 15 shows the overall carryover rate was averaged at 0.21% (5/2370). A total of 6 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 was averaged at 0.95% (4/422). For false positives in this subset, the carryover rate ranged from 0% to 2.16% across the three TIGRIS DTS Systems. These results demonstrate that carryover contamination is minimized on the TIGRIS DTS System.

Table 15: Summary of Overall TIGRIS DTS System Carryover

Instrument	# Valid Negative Tests	Total # GC False Positive Results	% GC False Positive Results	Confidence Intervals (95% CI)
TIGRIS 1	787	0[a]	0.00	0.00 - 0.38
TIGRIS 2	791	1[b]	0.13	0.00 - 0.70
TIGRIS 3	792	4[c]	0.51	0.14 - 0.29
All Instruments	2370	5	0.21	0.07 - 0.49

a. TIGRIS 1 had no false GC positive result directly following a high-target positive
b. TIGRIS 2 had one false GC positive result directly following a high-target positive
c. TIGRIS 3 had three false GC positive result directly following a high-target positive

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