Aptima® Neisseria gonorrhoeae Assay

For *in vitro* diagnostic use.

For U.S. Export only.

General Information

Intended Use

The Aptima® Neisseria gonorrhoeae (GC) Assay is an *in vitro* qualitative nucleic acid amplification test (NAAT) for the detection of ribosomal RNA (rRNA) from *Neisseria gonorrhoeae* (GC) to aid in the diagnosis of gonococcal urogenital disease using the Panther® System.

The assay may be used to test the following specimens from symptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; patient-collected vaginal swab specimens[1] and female and male urine specimens. The assay may be used to test 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. This assay is also intended for use with the testing of gynecological specimens, from both symptomatic and asymptomatic patients. These cervical specimens collected in the PreservCyt® Solution vials may be tested either pre- or post-Pap processing. Testing of post-Pap processed specimens is limited to specimens processed with the ThinPrep® 2000 System only.

[1] Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The Aptima® Multitest Swab Specimen Collection Kit is not for home use.

Summary and Explanation of the Test

Neisseria gonorrhoeae are one of the most common sexually transmitted infections (STIs) worldwide. In the United States alone, an estimated 1,568,000 new cases of GC infections were reported to the Centers for Disease Control in 2019 (1).

N. gonorrhoeae, a non-motile gram-negative diplococcus, is the causative agent of gonorrheal disease. 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) (6, 9).

Conventional diagnosis of GC infection requires isolation of the organism on selective media or the observation of diplococci in Gram stained smears (7). 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 (2, 8). Commonly used non-culture methods for GC detection include direct DNA probe tests and 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 (4). The 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 technologies (3, 5).

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 oligomer is the same for both assays, but the Aptima GC Assay detects a different region of the 16S rRNA subunit than the Aptima Combo 2 Assay and thus can be considered a suitable supplementary test to improve the PPV of Aptima Combo 2 Assay testing when recommended by local health guidelines.

Principles of the Procedure

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 use of a capture oligomer via target capture that utilizes magnetic microparticles. 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 microparticles, 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 Hologic® 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).

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. For additional specific warnings, precautions and procedures to control contamination for the Panther/Panther Fusion® System, consult the *Panther/Panther Fusion System Operator's Manual*.

D. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther/Panther Fusion System Operator's Manual* prior to performing the assay.

E. Only personnel adequately trained in the use of the Aptima GC Assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures

Laboratory Related

F. Use only supplied or specified disposable laboratory ware.

G. 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.

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

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

J. Dispose of all materials that have come in contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

K. Use good standard practices for molecular laboratories including environmental monitoring. See *Procedural Notes* for suggested Lab Contamination Monitoring Protocol for the Panther System.

Specimen Related

L. This assay has been tested using endocervical and male urethral swab specimens, PreservCyt Solution Pap specimens, vaginal swab specimens, and female and male urine specimens only. Performance with specimens other than those specified under *Specimen Collection and Storage* has not been evaluated.

Laboratories may validate other collection devices (10, 12).

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. The PreservCyt Solution has been validated as an alternative medium for testing with the Aptima GC Assay. PreservCyt Solution Pap specimens processed with instruments other

than the ThinPrep® Systems processor have not been evaluated for use with the Aptima GC Assay.

O. After urine has been added in the urine transport tube, the liquid level must fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.

P. 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.

Q. Avoid cross-contamination by discarding used materials without passing them over any other container.

R. 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.

S. 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 during specimen handling in the laboratory. Change gloves if they come in contact with specimen.

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

U. For PreservCyt Solution 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.

V. Upon piercing, liquid can discharge from Aptima® Specimen Transport Tube caps under certain conditions. Follow instructions in the *Panther System Test Procedure* to prevent this occurrence.

Assay Related

W. Cap and store reagents at the specified temperatures. The performance of the assay may be affected by use of improperly stored reagents. See *Reagent Storage and Handling Requirements* for more information.

X. Use Universal Precautions when handling controls.

Y. Avoid microbial and ribonuclease contamination of reagents.

Z. Do not use this kit or controls after its expiration date.

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

AB. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther System verifies reagent levels.

AC. Some reagents in this kit are labeled with hazard information.

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend at www.hologic.com/package-inserts.*

Canada Hazard Information

Selection Reagent
Boric Acid 1 - 5%
Triton X-100 1 - 5%

DANGER
H315 - Causes skin irritation.
H360FD - May damage fertility. May damage the unborn child.
P264 - Wash face, hands and any exposed skin thoroughly after handling.
P302 + P352 - IF ON SKIN: Wash with plenty of water and soap.
P321 - Specific treatment (see supplemental first aid instructions on the SDS.).
P332 + P313 - If skin irritation occurs: Get medical advice/attention.
P362 + P364 - Take off contaminated clothing and wash it before reuse.
P201 - Obtain special instructions before use.
P202 - Do not handle until all safety precautions have been read and understood.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P308 + P313 - IF exposed or concerned: Get medical advice/attention.
P405 - Store locked up.
P501 - Dispose of contents/container to an approved waste disposal plant.

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for reagents and controls.

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2ºC to 8ºC	N/A	N/A
Enzyme Reagent	2ºC to 8ºC	N/A	N/A
Probe Reagent	2ºC to 8ºC	N/A	N/A
Target Capture Reagent B	2ºC to 8ºC	N/A	N/A
Amplification Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Enzyme Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Probe Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	60 days
Selection Reagent	2ºC to 30ºC	2ºC to 30ºC	N/A
Target Capture Reagent	15ºC to 30ºC	15ºC to 30ºC	60 days
Positive Control, GC / Negative Control CT	2ºC to 8ºC	N/A	Single Use Vial
Positive Control, CT/ Negative Control GC	2ºC to 8ºC	N/A	Single Use Vial

B. If the Selection Reagent is stored refrigerated, let it come to room temperature before placing on the Panther System.

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

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

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

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

G. Avoid cross-contamination during reagent handling and storage. Recap all reconstituted reagents with new reagent caps each time prior to storage.

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

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

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

K. Do not freeze the reagents.

Specimen Collection and Storage

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

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 Solution Pap specimens. Performance with specimens other than those collected with the following specimen collection kits has not been evaluated:

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

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 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.
   b. If longer storage is needed, freeze urogenital specimens in the swab specimen transport tube within 7 days of collection at –20°C to –70°C to allow testing up to 12 months after collection.

2. Urine Specimens
   a. Maintain urine specimen at 2°C to 30°C after collection and transfer to the Aptima® Urine Specimen Transport Tube within 24 hours of collection. Transport to the lab in the primary collection container or the transport tube at 2°C to 30°C. Store at 2°C to 30°C and test the processed urine specimens with the Aptima GC Assay within 30 days of collection.
   b. If longer storage is needed, freeze urine specimens in the Aptima Urine Specimen Transport Tube within 7 days of collection at –20°C to –70°C to allow testing up to 12 months after collection.
3. PreservCyt Solution Pap Specimens
   a. PreservCyt Solution Pap specimens intended for GC testing must be processed for cytology and/or transferred to an Aptima Specimen Transfer Tube within 30 days of collection when stored at 2°C to 30°C.
   b. If the ThinPrep Aliquot Removal procedure will be used, refer to the *ThinPrep Systems Processor Operator's Manual—Addendum* for instructions on aliquot removal. Transfer 1 mL of the removed aliquot into an Aptima Specimen Transfer Tube according to the instructions in the Aptima Specimen Transfer Kit and Aptima Transfer Solution package insert.
   c. If testing the specimen after processing using the ThinPrep Systems processor, process the PreservCyt Solution Pap specimen in accordance with the *ThinPrep Systems Processor Operator's Manual* and the Aptima Specimen Transfer Kit and Aptima Transfer Solution 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 and Aptima Transfer Solution package insert.
   d. Once the PreservCyt Solution 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 specimen within 7 days of transfer to the Aptima Specimen Transfer Tube at –20°C to –70°C to allow testing up to 12 months after transfer.

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, foil barrier, or cap.

Note: *Any condition resulting in loss or evaporation of media during transport, handling, or storage may impact the ability to pipette multiple aliquots.*

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.
4. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination**.

Note: *Specimens must be shipped in accordance with applicable national and international transportation regulations.*

Panther System

Reagents for the Aptima GC Assay are listed below for the Panther System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima Neisseria gonorrhoeae Assay Kit, 100 tests (2 boxes and 1 Controls kit) (Cat. No. 302927)

Aptima Neisseria gonorrhoeae Assay Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
TCR-B	**Target Capture Reagent B** *Non-infectious nucleic acids in a buffered solution containing < 5% detergent.*	1 x 0.30 mL

Aptima Neisseria gonorrhoeae Assay Room Temperature Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	**Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 11.9 mL
ER	**Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 6.3 mL
PR	**Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 15.2 mL
S	**Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 43.0 mL
TCR	**Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 26.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

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

Symbol	Component	Quantity
PGC/ NCT	**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
PCT/ NGC	**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

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

Materials Required but Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

	Cat. No.
Panther System	303095
Panther Fusion System	PRD-04172
Panther System, Continuous Fluid and Waste	PRD-06067
Aptima® Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima® Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther® Waste Bag Kit	902731
Panther® Waste Bin Cover	504405
Or Panther® Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable *Not all products are available in all regions. Contact your representative for region-specific information*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima Specimen Transfer Kit *for use with specimens in PreservCyt Solution*	301154C
Aptima Specimen Transfer Kit — printable *for use with specimens in PreservCyt Solution*	PRD-05110
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041

	Cat. No.
Aptima Urine Specimen Collection Kit for Male and Female Urine Specimens	301040
Aptima® Urine Specimen Transport Tubes for Male and Female Urine Specimens	105575
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable gloves	—
Aptima penetrable caps	105668
Replacement non-penetrable caps	103036A
Replacement caps for the 100-test kits	—
Amplification, Enzyme, and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
TCR and Selection reagent	*501604 (100 caps)*

Optional Materials

	Cat. No.
Aptima® Controls Kit	301110
Hologic® Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Tube Rocker	—
Lint-free Wipes	—
Plastic-backed Bench Covers	—

Panther System Test Procedure

Note: *See the Panther/Panther Fusion System Operator's Manual for additional Panther System procedural information.*

A. Work Area Preparation

1. Clean work surfaces where reagents 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 will be prepared with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (Step A.1).
3. Clean any pipettors. Use cleaning procedure described above (Step A.1).

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent reconstitution should be performed prior to beginning any work on the Panther System.*

1. Prior to testing, Amplification, Enzyme, and Probe Reagents must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate

reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.

a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.

b. Pair each reconstitution solution with its lyophilized reagent. Before attaching the reconstitution collar, ensure that the reconstitution solution and reagent have matching label symbols.

c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired. Label caps of reconstitution solution bottles.

d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 1, Step 1).

e. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.

f. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 1, Step 2).

g. Slowly invert the assembled bottles. Allow the solution to drain from the reconstitution solution bottle into the glass vial (Figure 1, Step 3).

h. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle. (Figure 1, Step 4).

i. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the reconstitution solution bottle.

j. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

k. Recap the plastic bottle with either the saved labeled cap that corresponds to the reagent or new cap. Do not mismatch caps. Record the operator initials and reconstitution date on the label (Figure 1, Step 7).

l. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

m. Thoroughly mix each reagent by gently inverting prior to loading onto the Panther System.

Option: *Additional mixing of the Amplification, Enzyme and Probe Reagents is allowed by placing recapped plastic bottles on a tube rocker set at a moderate speed and tilt for a minimum of 5 minutes. Ensure reagents are thoroughly mixed.*

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

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

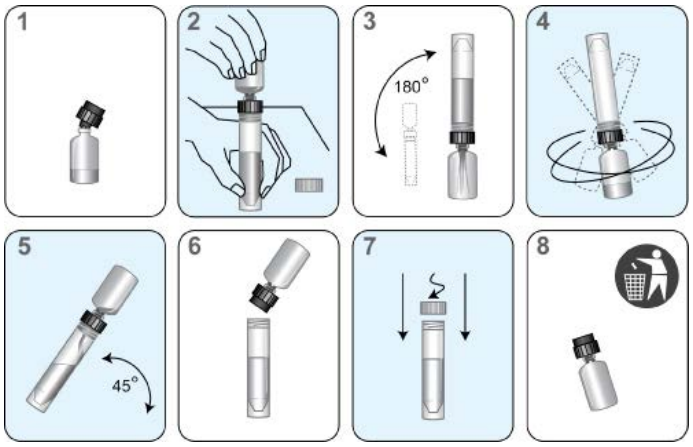

Figure 1. Panther System Reconstitution Process

2. Prepare the working Target Capture Reagent (wTCR)
   a. Pair the appropriate bottles of TCR and TCR-B.
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the bottle of TCR-B and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the TCR-B bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label.
   g. Discard the TCR-B bottle and cap.
3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure that it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

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

C. Reagent Preparation for Previously Reconstituted Reagents

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

Option: *The reconstituted Amplification, Enzyme and Probe Reagents capped plastic bottles may be placed on a tube rocker set at a moderate speed and tilt for a minimum of 25 minutes until reagents reach room temperature and are thoroughly mixed.*

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

3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

D. Control Preparation

1. Remove the controls from storage (2°C to 8°C) and allow the controls to reach room temperature (15°C to 30°C) prior to processing.

E. Specimen Handling

1. Visually confirm that each specimen tube meets one of the following criteria.
   a. The presence of a single pink Aptima collection swab in a multitest swab specimen transport tube.
   b. A final volume of urine between the black fill lines of a urine specimen transport tube.
   c. The presence of a single blue Aptima collection swab in a unisex swab specimen transport tube.
   d. The absence of a swab in the Aptima Specimen Transport Tube for PreservCyt Solution Pap specimens.
2. Allow the specimens to reach room temperature (15°C to 30°C) prior to processing.

Note: *Prior to testing and/or to resolve suspected specimen related invalid results, specimens may be vortexed at high speed for a minimum of 3 minutes, followed by low speed vortexing for 1 minute (to draw the fluid down into the tube).*

3. Inspect specimen tubes before loading into the 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 3a–c may result in liquid discharge from the specimen tube cap.*

Note: *Up to 5 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 5 aliquots from the specimen tube can lead to processing errors.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes*.

Note: *Make sure that the appropriately sized reagent racks and TCR adapters are used.*

2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Panther System software using the AGC Assay protocol, one pair of controls is required. The Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control CT tubes can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Patient specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of controls is currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated assay reagent kit up to 24 hours **unless:**
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

B. Temperature

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

C. Glove Powder

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

D. Lab Contamination Monitoring Protocol for the Panther System

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

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

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 if using the Aptima Unisex Swab Specimen Collection Kit) from its packaging, wet the swab in the Aptima® Specimen Transport Medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.

6. Repeat Steps 2 to 5 for each area to be swabbed.
7. Test samples with the Aptima GC Assay on the Panther System.
8. Further investigation should be performed if any samples yield a positive result.

If the results are GC positive or equivocal, see *Test Interpretation — QC/Patient Results*. For additional Panther System-specific contamination monitoring information, contact Hologic Technical Support.

Test Interpretation — QC/Patient Results

A. Test Interpretation

Assay test results are automatically interpreted by the Panther System software using the Aptima GC Assay 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. Report the first valid test result. If the result is invalid upon retest, a new specimen should be collected

Test Interpretation	Total RLU (x1,000)
Negative	0* to < 50
Equivocal	50 to < 100
Low RLU Positive[1, 2]	100 to < 2,000
Positive[1]	2,000 to < 12,000
Invalid	0* or > 12,000

*A zero (0 x 1,000) RLU result on the run report represents a value between zero and 999 RLU. RLU values less than 690 on the Panther System will be reported as invalid.

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

[2]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 Negative Control for GC, which is labeled “CONTROL + CT PCT / CONTROL – GC NGC,” and the 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. Correct preparation of specimens is confirmed visually by the presence of a single Aptima collection swab in a swab specimen transport tube, a final volume of urine in between the black fill lines of a urine specimen transport tube, or the absence of a swab in an Aptima Specimen Transfer Tube for PreservCyt Solution Pap specimens.

The Controls must produce the following test results:

Control	Total RLU (x1,000)	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 1,000) RLU result on the run report represents a value between zero and 999 RLU. RLU values less than 690 on the Panther System will be reported as invalid.

1. The Panther System software, using the Aptima GC Assay protocol, automatically evaluates the controls according to the above criteria and the results will be reflected in the results report.
2. If the Run Status is INVALID, all test results in the same run are invalid and must not be reported.
3. Each laboratory should implement appropriate control procedures to satisfy the requirements of local regulations.
4. Negative controls may not be effective in monitoring random carryover. See *Analytical Performance* for results from a high-target analytical carryover study that was performed to demonstrate control of carryover on the Panther System.

C. Specimen Preparation Control (optional)

The Negative Control for GC, which is labeled “CONTROL + CT PCT / CONTROL – GC NGC,” and the 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*, *Patient Test 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 Solution 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 — QC/Patient Results* 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 Assay 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 Pap specimen must be collected before the endocervical swab specimen.

Limitations

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

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

C. 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.

D. Urine, vaginal swab, and PreservCyt Solution 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.

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, proper specimen collection techniques are necessary. Refer to package insert of the appropriate 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 for detecting GC is 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 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. Performance of the Aptima Specimen Transfer Kit was not evaluated for testing the same PreservCyt Solution Pap specimen both before and after ThinPrep Pap processing.

N. PreservCyt Solution Pap specimens processed with instruments other than the ThinPrep processor have not been evaluated.

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

P. The patient-collected vaginal swab specimen application is limited to clinical settings where support/counseling is available to explain the procedures and precautions.

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

R. The performance of the Aptima GC Assay has not been evaluated in adolescents less than 14 years of age.

S. 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.

T. The performance of the Panther System has not been evaluated at altitudes above 2,000 m (6,561 feet).

U. There is no evidence of degradation of nucleic acids in PreservCyt Solution. If a PreservCyt Solution 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 STM, 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.

V. Customers must independently validate an LIS transfer process.

Clinical Study Results

The performance of the Aptima GC Assay was established in three 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 second clinical investigation established the sensitivity, specificity, and predictive values of the Aptima GC Assay using PreservCyt Solution (component of the ThinPrep 2000 System).

The initial clinical investigations to establish the sensitivity, specificity and predictive values of the Aptima GC Assay were completed using a semi-automated DTS® System. The assay was then migrated to a fully automated Tigris® DTS System (without any changes to assay formulation) using clinical comparability studies. Lastly, clinical comparability studies were used to migrate the Aptima GC Assay from Tigris DTS to its current system of use, the Panther System. Data from the initial studies using the DTS or Tigris DTS Systems may be shown herein to support establishment of the assay performance, although current use of these systems is no longer supported by the manufacturer.

In the third clinical investigation, the clinical performance of the Aptima GC Assay was evaluated in sexually active male and female subjects at least 14 years of age with or without symptoms of STIs. This study evaluated patient-collected vaginal swab and urine specimens tested using the Panther System.

Expected Values

The prevalence of GC in patient populations depends on risk factors such as age, lifestyle, the presence or absence of symptoms, and the sensitivity of the test used to detect infections. A summary of the positivity of GC in North America, by specimen type as determined by the Aptima GC Assay is shown in Tables 1a and 1b for two clinical investigations. Table 1c summarizes positivity of *N. gonorrhoeae* for the Aptima GC Assay on the Panther System as determined by an additional clinical investigation.

Table 1a: Positivity of GC by Clinical Site and Overall as Determined by Aptima GC Assay Results on the DTS System

	% (#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	(214/1,318)	14.3	(189/1,322)	5.9	(85/1,452)	4.9	(72/1,459)	5.8	(83/1,434)	5.8	(84/1,458)

MS = male urethral swab; **MU** = male urine; **FS** = female endocervical swab; **FU** = female urine; **PVS** = patient-collected vaginal swab; **CVS** = clinician-collected vaginal swab; **N/A** = not available.

Table 1b: Positivity of GC by Clinical Site and Overall as Determined by Aptima GC Assay Results on the DTS System Using PreservCyt Solution Pap 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	(16/1,647)

Table 1c: Positivity of GC as Determined by Aptima GC Assay in Patient-collected Vaginal Swab, Female Urine, and Male Urine Samples by Clinical Site

Site	Positivity % (# positive/# tested with valid non-equivocal results)		
	PVS	FU	MU
1	14.3 (3/21)	13.6 (3/22)	21.7 (38/175)
2	1.3 (5/383)	1.3 (5/385)	0.8 (3/373)
3	0 (0/75)	0 (0/74)	0 (0/61)
4	0 (0/5)	0 (0/5)	0 (0/13)
5	2.0 (5/254)	2.0 (5/250)	8.3 (34/409)
6	2.0 (10/494)	2.1 (10/484)	9.4 (29/307)
7	2.0 (5/246)	1.6 (4/245)	5.3 (12/225)
8	0 (0/95)	0 (0/97)	0 (0/32)
9	0.3 (1/313)	0 (0/261)	0 (0/218)
10	4.3 (11/255)	4.0 (10/253)	11.0 (10/91)
11	0 (0/96)	0 (0/91)	0 (0/54)
All	1.8 (40/2,237)	1.7 (37/2,167)	6.4 (126/1,958)

FU = female urine; **MU** = male urine; **PVS** = patient-collected vaginal swab.

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 on the DTS System are shown in Table 2a. 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 the Aptima GC Assay on the DTS System are 97.6% and 99.3%, respectively (Table 2a). 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 6a for each clinical site and overall. The actual PPV and NPV for PreservCyt Solution Pap specimens using the Aptima GC Assay on the DTS System are shown in Table 6b.

Table 2a: Positive and Negative Predictive Values for Hypothetical Prevalence Rates in North America on the DTS System

Hypothetical Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
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

The estimated PPV and NPV of the Aptima GC Assay on the Panther System across different hypothetical prevalence rates are shown for each specimen type in Table 2b. For each specimen type, the PPV and NPV are derived for different hypothetical prevalence

rates using the overall sensitivity and specificity estimates from the multi-center clinical study (see Table 10).

Table 2b: Positive and Negative Predictive Values for Hypothetical Prevalence Rates in North America on the Panther System

Specimen Type		Hypothetical Prevalence						
		1%	2%	5%	10%	15%	20%	25%
PVS	PPV (%)	91.3	95.5	98.2	99.1	99.5	99.6	99.7
	NPV (%)	99.9	99.9	99.7	99.4	99.1	98.8	98.4
FU	PPV (%)	95.2	97.6	99.0	99.5	99.7	99.8	99.8
	NPV (%)	99.9	99.8	99.6	99.2	98.7	98.1	97.5
MU	PPV (%)	94.8	97.4	99.0	99.5	99.7	99.8	99.8
	NPV (%)	100	100	99.9	99.8	99.7	99.6	99.5

FU = female urine; **MU** = male urine; **NPV** = negative predictive value; **PPV** = positive predictive value; **PVS** = patient-collected vaginal swab.

Aptima GC Assay on the DTS System RLU Distribution

Figure 2 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.

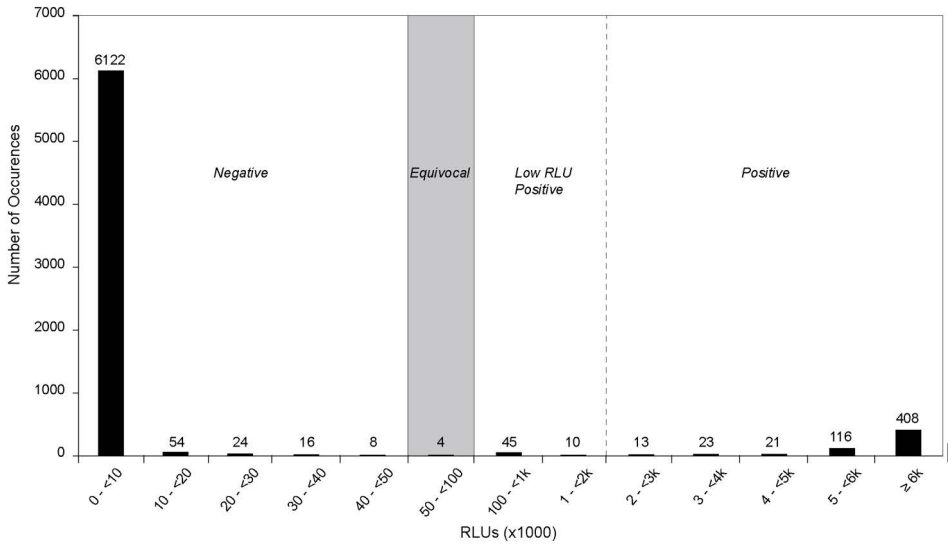

Figure 2. Frequency of RLU Distribution for the Aptima GC Assay on the DTS System

Table 3: Aptima GC Assay RLU Distribution on the DTS System

	RLUs (x 1,000)												
	0–<10	**10–<20**	**20–<30**	**30–<40**	**40–<50**	**50–<100**	**100–<1,000**	**1,000–<2,000**	**2,000–<3,000**	**3,000–<4,000**	**4,000–<5,000**	**5,000–<6,000**	**≥6,000**
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	**6,122**	**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.

Clinical Performance

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

Clinician-collected endocervical, vaginal and male urethral swab, patient-collected vaginal swab, and male and female urine specimens were collected from 2,787 symptomatic and asymptomatic, male and female subjects attending STI, OB/GYN, 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 6a shows the Aptima GC Assay sensitivity, specificity, and predictive values compared to patient infected status for each clinical site and overall. Tables 6c–6g 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% CI)		Specificity (95% CI)	
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	1,321	180	9[d]	1,130	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	1,440	72	13[g]	1,353	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	1,449	69	3[j]	1,371	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[l]	749	1	98.1	(89.9–100)	99.1	(98.1–99.6)
		Asymptomatic	637	21	4[m]	611	1	95.5	(77.2–99.9)	99.3	(98.3–99.8)
		All	1,446	73	11[n]	1,360	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; **CI** = confidence interval.

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.

PreservCyt Solution Pap Specimen Clinical Specimen Study

A prospective multi-center clinical study was conducted to evaluate the use of the PreservCyt Solution (a component of the ThinPrep 2000 System) as an alternative medium for gynecological specimens for the detection of GC 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 STI clinics were enrolled and evaluated in the clinical study. Of these subjects, 1,288 were asymptomatic subjects and 359 were symptomatic subjects (Table 6g). Subjects were enrolled from sites with GC prevalence that ranged from 0.0% to 5.0% (Table 6b).

Two specimens were collected from each eligible subject: one PreservCyt Solution Pap specimen and one endocervical swab specimen. PreservCyt Solution 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 5a by specimen collection site and overall.

PreservCyt Solution Pap specimens were processed in accordance with the *ThinPrep 2000 Processor Operator's Manual* and Aptima Specimen Transfer Kit and Aptima Transfer Solution Kit package insert. After processing the PreservCyt Solution 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 Solution 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 6g summarizes the frequency of test outcomes for the endocervical swab specimens tested with the Aptima Combo 2 Assay and Aptima GC Assay.

Table 5b 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% (1,630/1,634). In symptomatic and asymptomatic subjects, specificities were 99.4% (350/352) and 99.8% (1,280/1,282), respectively.

Table 6b 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 5a: Distribution of Cervical Sampling Device Used for PreservCyt Solution Pap Specimens

Cervical Sampling Device Used	Clinical Collection Site						Total
	1	2	3	4	5	6	
Spatula/Cytobrush	0	124	475	287	57	364	1,307
Broom-Type Device	100	0	0	0	240	0	340

Table 5b: Sensitivity and Specificity of the Aptima GC Assay Relative to Patient Infected Status by Symptom Status and Overall for PreservCyt Solution 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	1,275		
	Total	6	0	6	1,276		
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	1,625		
	Total	13	0	6	1,628		

CI = confidence interval.

+/+ = 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 6a: 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% CI)		Specificity (95% CI)		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 6a: 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% CI)		Specificity (95% CI)		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; **Prev** = prevalence; **CI** = confidence interval; **PPV** = positive predictive value; **NPV** = negative predictive value; **NA** = not available.

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

Site	Aptima GC PreservCyt Solution Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	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	1,625					
	Total	13	0	6	1,628					

Prev = prevalence; **PPV** = positive predictive value; **NPV** = negative predictive value; **CI** = confidence interval; **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 6c: Symptomatic Male Urethral 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	Total
	MS	MU	MS	MU	MS	
Infected	+	+	+	+	+	164
Infected	+	+	+	+	-	1
Infected	+	+	+	-	+	3
Infected	+	+	=	+	+	1
Infected	+	-	+	+	+	2
Infected	+	-	+	-	+	1
Non-infected	+	-	-	-	+	2
Non-infected	+	-	-	-	-	1
Non-infected	-	+	-	-	+	1
Non-infected	-	-	+	-	-	1
Non-infected	-	-	-	+	-	2
Non-infected	-	-	-	-	+	3
Non-infected	-	-	-	-	+	2
Non-infected	-	-	-	-	-	386
Non-infected	-	-	-	-	=	1
Non-infected	-	-	-	N/A	-	1
Non-infected	-	-	-	=	-	1
Non-infected	-	-	=	-	-	1
Non-infected	=	-	-	-	+	2
Total						**576**

N/A = specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing. **MS** = symptomatic male urethral swab; **MU** = male urine.

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

Patient Infected Status	NAAT 1 (Aptima Combo 2 Assay)		NAAT 2		Aptima GC Assay	Symptom Status		Total
	MS	MU	MS	MU	MU	Sym	Asym	
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	1,077
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**	**1,321**

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing. **Sym** = symptomatic; **Asym** = asymptomatic; **MS** = male urethral swab; **MU** = male urine.

Table 6e: 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		Total
	FS	FU	FS	FU	FS	FU	Sym	Asym	
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	1,307
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**	**1,451**

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing. **Sym** = symptomatic; **Asym** = asymptomatic; **FS** = female endocervical swab; **FU** = female urine.

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

Patient Infected Status	NAAT 1 (Aptima Combo 2 Assay)		NAAT 2		Aptima GC Assay		Symptom Status		Total
	FS	FU	FS	FU	PVS	CVS	Sym	Asym	
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	1,273
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**	**1,451**

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing. **Sym =** symptomatic; **Asym =** asymptomatic; **FS** = female endocervical swab; **FU** = female urine; **PVS** = patient-collected vaginal swab; **CVS** = clinician-collected vaginal swab.

Table 6g: PreservCyt Solution Clinical Study (Patient Infected Status Results for Endocervical Swab Specimens)

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	1,276
Non-Infected	Negative	Positive	0	5
Non-Infected	Equivocal	Positive	0	1
Total			**359**	**1,288**

RLU Distribution of Aptima Controls

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

Table 7: 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 Solution Pap Studies

Control	Statistics	RLU (x1,000)	
		Swab and Urine Specimen Clinical Study	PreservCyt Solution Pap Specimen Clinical Study
Positive Control, GC / Negative Control, CT	N	193	218
	Mean	5,048	4,561
	SD	1,071	1,295
	Maximum	6,765	6,791
	75th Percentile	5,763	5,450
	Median	5,175	4,859
	25th Percentile	4,645	3,804
	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

RLU = relative light units; **SD** = standard deviation.

Note: The RLU value reported by the software was the bases for analysis. The reported RLU value is the total measured RLU divided by 1,000 with the digits after the decimal point truncated.

Precision Study

Aptima GC Assay precision (i.e., reproducibility) was evaluated at two external clinical sites and at Hologic. 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 STM spiked with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Table 8 presents the precision RLU data in terms of Mean, Standard Deviation, Coefficient of Variation (CV), and percent agreement with expected results for calculations of between-site, between-operator, between-lot, between-run, and within-run variability.

Table 8: 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 (x1,000)	% Agrmt.	Within-Run		Between-Site		Between-Lot		Between-Operator		Between-Run	
				SD RLU (x1,000)	CV (%)	SD RLU (x1,000)	CV (%)	SD RLU (x1,000)	CV (%)	SD RLU (x1,000)	CV (%)	SD RLU (x1,000)	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	5,574.4	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	6,502.6	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	6,786.0	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; **RLU** = relative light unit; **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 (11).

PreservCyt Solution Pap specimen within-laboratory precision with the Aptima GC Assay was determined by spiking PreservCyt vials with 20 GC CFU (Colony Forming Units) 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 9.

Table 9: Aptima GC Assay Within-Laboratory Precision Data for PreservCyt Solution Pap Specimen 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 (x1,000)	Within-Operator		Between-Day		Between-Operator		Total	
							SD (x1,000)	CV (%)	SD (x1,000)	CV (%)	SD (x1,000)	CV (%)	SD (x1,000)	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	1,211.1	1,031.3	85.2	169.8	14.0	150.4	12.4	1,056.0	87.2
C	500	50	140	140	100	5,636.8	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

SD = standard deviation; **CV (%)** = percent coefficient of variation; **% Agrmt.** = percent agreement; **N/A** = not applicable for negative panel members; **Operator** = run.

* 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 (11). Samples with discordant results were included in the signal variability analysis.

Panther System Clinical Performance

Clinical Study

A prospective, multicenter clinical study was conducted to establish the clinical performance characteristics of the Aptima GC Assay on the Panther System. Specimens were collected from 4,413 symptomatic and asymptomatic women and men enrolled at 11 geographically and ethnically diverse US clinical sites, including obstetrics and gynecology, family planning, and STI clinics. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. One hundred ninety (190) enrolled subjects were not evaluable (28 were withdrawn and 162 did not have at least one specimen with a valid non-excluded Aptima result and a conclusive infected status). Of the 4,223 evaluable subjects, 2,264 were women and 1,959 were men. The average age among evaluable study subjects was 34.5 years (range = 14 to 84 years). Symptoms were reported in 45.6% (1,927/4,223) of the evaluable subjects.

Up to 5 specimens were collected from each female subject (1 first-catch urine, 4 patient-collected vaginal swab, in that order) and 1 first-catch urine specimen was collected from each male subject. All specimens were collected by the subject at the clinical sites.

Specimens were tested with the Aptima GC Assay on the Panther System. Specimens with initial equivocal or invalid Aptima GC Assay results or instrument processing errors were retested, volume permitting; valid retest results were included in the performance analyses. Patient-collected vaginal swabs and male and female urine specimens were tested with up to 3 FDA-cleared NAATs to establish the specimen-specific patient infected status (PIS) as follows:

- Male urine PIS was derived from male urine specimens
- Female urine PIS was derived from female urine specimens
- Vaginal swab PIS was derived from vaginal swab and female urine specimens

Performance of the Aptima GC Assay was estimated relative to the specimen-specific PIS for each of the specimen types.

Of the specimens collected, 6,556 were processed in valid Aptima GC Assay runs, including 218 (3.3%) that had to be retested due to initial invalid results. Overall, 6,513 (99.3%) had final valid results, and 43 (0.7%) had final invalid results and were excluded from the analyses. A total of 6,362 specimens from 4,222 evaluable subjects were included in the analyses comparing Aptima GC Assay results to the PIS: 2,237 patient-collected vaginal swab, 2,167 female urine, and 1,958 male urine specimens. Four specimens with final GC equivocal results were excluded from the performance analyses.

Performance Results

Performance characteristics of the Aptima GC Assay were estimated for each specimen type. Table 10 shows the sensitivity, specificity, PPV, and NPV of the Aptima GC Assay on

the Panther System and the prevalence of *GC* (based on the specimen-specific PIS) in each specimen type by symptom status and overall.

Table 10: Performance Characteristics of the Aptima GC Assay Female Patient-collected Vaginal Swab, and Male and Female Urine Specimens by Symptom Status

Specimen Type	Symptom Status	n	TP	FP[1]	TN	FN[2]	Prev %	Sensitivity % (95% CI)[3]	Specificity % (95% CI)[3]	PPV % (95% CI)[4]	NPV % (95% CI)[4]
PVS	Sym	1,086	24	1[a]	1,060	1[a]	2.3	96.0 (80.5–99.3)	99.9 (99.5–100)	96.0 (81.7–99.9)	99.9 (99.5–100)
	Asym	1,151	14	1[b]	1,135	1[b]	1.3	93.3 (70.2–98.8)	99.9 (99.5–100)	93.3 (72.6–99.8)	99.9 (99.6–100)
	All	2,237	38	2	2,195	2	1.8	95.0 (83.5–98.6)	99.9 (99.7–100)	95.0 (84.5–99.6)	99.9 (99.7–100)
FU	Sym	1,043	25	0	1,018	0	2.4	100 (86.7–100)	100 (99.6–100)	100 (87.2–100)	100 (99.7–100)
	Asym	1,124	11	1[c]	1,109	3[c]	1.2	78.6 (52.4–92.4)	99.9 (99.5–100)	91.7 (66.0–99.7)	99.7 (99.4–100)
	All	2,167	36	1	2,127	3	1.8	92.3 (79.7–97.3)	100 (99.7–100)	97.3 (87.2–99.9)	99.9 (99.6–100)
MU	Sym	825	105	1[d]	717	2[d]	13.0	98.1 (93.4–99.5)	99.9 (99.2–100)	99.1 (95.1–100)	99.7 (99.0–100)
	Asym	1,133	20	0	1,113	0	1.8	100 (83.9–100)	100 (99.7–100)	100 (84.4–100)	100 (99.7–100)
	All	1,958	125	1	1,830	2	6.5	98.4 (94.4–99.6)	99.9 (99.7–100)	99.2 (95.8–100)	99.9 (99.6–100)

Sym = asymptomatic; **Asym** = asymptomatic; **TP** = true positive; **FP** = false positive; **TN** = true negative; **FN** = false negative; **Prev** = prevalence; **CI** = confidence interval; **PVS** = patient-collected vaginal swab; **FU** = female urine; **MU** = male urine; **PPV** = positive predictive value; **NPV** = negative predictive value.

[1]Specimens of the same type were also tested by an alternative *N. gonorrhoeae* NAAT assay with the following results (# positive results / # samples tested): [a]0/1; [b]0/1; [c]0/1; [d]1/1.

[2]Specimens of the same type were also tested by an alternative *N. gonorrhoeae* NAAT assay with the following results (# negative results / # samples tested); [a]0/1; [b]0/1; [c]1/3; [d]1/2.

[3]Score CI.

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

Table 11 shows the sensitivity, specificity, PPV, and NPV of the Aptima GC Assay on the Panther System and the prevalence of *N. gonorrhoeae* (based on the specimen-specific PIS) in each specimen type by collection site. Prevalence varied across collection sites, as expected.

Table 11: Performance Characteristics of the Aptima GC Assay by Collection Site

Specimen Type	Site	N	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
PVS	**1**	21	3	0	18	0	14.3	100 (43.9–100)	100 (82.4–100)	100 (46.2–100)	100 (89.5–100)
	2	383	5	0	378	0	1.3	100 (56.6–100)	100 (99.0–100)	100 (57.7–100)	100 (99.3–100)
	3	75	0	0	75	0	0.0	NC	100 (95.1–100)	NC	100 (NC)
	4	5	0	0	5	0	0.0	NC	100 (56.6–100)	NC	100 (NC)
	5	254	5	0	249	0	2.0	100 (56.6–100)	100 (98.5–100)	100 (57.7–100)	100 (99.0–100)
	6	494	9	1	483	1	2.0	90.0 (59.6–98.2)	99.8 (98.8–100)	90.0 (63.1–99.6)	99.8 (99.1–100)
	7	246	4	1	241	0	1.6	100 (51.0–100)	99.6 (97.7–99.9)	80.0 (39.9–99.4)	100 (99.0–100)
	8	95	0	0	95	0	0.0	NC	100 (96.1–100)	NC	100 (NC)
	9	313	1	0	312	0	0.3	100 (20.7–100)	100 (98.8–100)	100 (6.4–100)	100 (99.7–100)
	10	255	11	0	243	1	4.7	91.7 (64.6–98.5)	100 (98.4–100)	100 (76.3–100)	99.6 (98.1–100)
	11	96	0	0	96	0	0.0	NC	100 (96.2–100)	NC	100 (NC)

Table 11: Performance Characteristics of the Aptima GC Assay by Collection Site (continued)

Specimen Type	Site	N	TP	FP	TN	FN	Prev %	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
FU	**1**	22	3	0	19	0	13.6	100 (43.9–100)	100 (83.2–100)	100 (46.1–100)	100 (90.0–100)
	2	385	5	0	379	1	1.6	83.3 (43.6–97.0)	100 (99.0–100)	100 (59.6–100)	99.7 (99.0–100)
	3	74	0	0	74	0	0.0	NC	100 (95.1–100)	NC	100 (NC)
	4	5	0	0	5	0	0.0	NC	100 (56.6–100)	NC	100 (NC)
	5	250	5	0	245	0	2.0	100 (56.6–100)	100 (98.5–100)	100 (57.7–100)	100 (98.9–100)
	6	484	9	1	473	1	2.1	90.0 (59.6–98.2)	99.8 (98.8–100)	90.0 (63.1–99.6)	99.8 (99.1–100)
	7	245	4	0	241	0	1.6	100 (51.0–100)	100 (98.4–100)	100 (52.2–100)	100 (99.0–100)
	8	97	0	0	97	0	0.0	NC	100 (96.2–100)	NC	100 (NC)
	9	261	0	0	261	0	0.0	NC	100 (98.5–100)	NC	100 (NC)
	10	253	10	0	242	1	4.3	90.9 (62.3–98.4)	100 (98.4–100)	100 (74.6–100)	99.6 (98.2–100)
	11	91	0	0	91	0	0.0	NC	100 (95.9–100)	NC	100 (NC)
MU	**1**	175	38	0	137	0	21.7	100 (90.8–100)	100 (97.3–100)	100 (91.3–100)	100 (97.5–100)
	2	373	3	0	370	0	0.8	100 (43.9–100)	100 (99.0–100)	100 (44.4–100)	100 (99.4–100)
	3	61	0	0	61	0	0.0	NC	100 (94.1–100)	NC	100 (NC)
	4	13	0	0	13	0	0.0	NC	100 (77.2–100)	NC	100 (NC)
	5	409	34	0	374	1	8.6	97.1 (85.5–99.5)	100 (99.0–100)	100 (90.5–100)	99.7 (98.6–100)
	6	307	28	1	278	0	9.1	100 (87.9–100)	99.6 (98.0–99.9)	96.6 (83.5–99.9)	100 (98.8–100)
	7	225	12	0	213	0	5.3	100 (75.8–100)	100 (98.2–100)	100 (76.6–100)	100 (98.6–100)
	8	32	0	0	32	0	0.0	NC	100 (89.3–100)	NC	100 (NC)
	9	218	0	0	218	0	0.0	NC	100 (98.3–100)	NC	100 (NC)
	10	91	10	0	80	1	12.1	90.9 (62.3–98.4)	100 (95.4–100)	100 (74.9–100)	98.8 (94.6–100)
	11	54	0	0	54	0	0.0	NC	100 (93.4–100)	NC	100 (NC)

TP = true positive; **FP** = false positive; **TN** = true negative; **FN** = false negative; **Prev** = prevalence; **CI** = confidence interval; **PPV** = positive predictive value; **NPV** = negative predictive value; **PVS** = patient-collected vaginal swab; **FU** = female urine; **MU** = male urine; **NC** = not calculable.

[1] Score CI.

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

Neisseria gonorrhoeae Infected Status Tables

The frequency of test outcomes from reference NAAT and investigational Panther System testing is summarized in Table 12a and Table 12b.

Table 12a: N. gonorrhoeae Infected Status for Female and Male Urine Specimens

Specimen Type	Patient Infected Status	NAAT 1	NAAT 2	NAAT 3	AGC Assay	Symptom Status	
						Symptomatic	Asymptomatic
FU	Infected	+	+	N/A	+	21	10
	Infected	+	+	N/A	-	0	2
	Infected	+	NR	+	+	1	0
	Infected	-	+	+	+	2	0
	Infected	-	+	+	-	0	1
	Infected	NR	+	+	+	1	1
	Non-infected	-	+	-	-	0	2
	Non-infected	-	-	N/A	+	0	1
	Non-infected	-	-	N/A	-	981	1,077
	Non-infected	-	NR	-	-	1	1
	Non-infected	NR	-	-	-	36	29
MU	Infected	+	+	N/A	+	97	19
	Infected	+	+	N/A	-	2	0
	Infected	+	NR	+	+	1	0
	Infected	-	+	+	+	2	1
	Infected	NR	+	+	+	5	0
	Non-infected	+	-	-	+	1	0
	Non-infected	-	+	-	-	1	2
	Non-infected	-	-	N/A	-	689	1,079
	Non-infected	-	-	N/A	=	0	1
	Non-infected	-	NR	-	-	1	0
	Non-infected	NR	-	-	-	26	32

AGC Assay = Aptima Neisseria gonorrhoeae Assay; **FU** = female urine; **MU** = male urine; **N/A** = not applicable; **NR** = no result. Note: The equal symbol (=) represents a final equivocal result.

Table 12b: N. gonorrhoeae Infected Status for Patient-collected Vaginal Swab Specimens

Patient Infected Status	NAAT 1		NAAT 2		AGC Assay	Symptom Status	
	PVS	FU	PVS	FU		Symptomatic	Asymptomatic
Infected	+	+	+	+	+	20	12
Infected	+	+	+	+	-	0	1
Infected	+	+	+	NR	+	1	0
Infected	+	-	+	+	+	1	0
Infected	+	-	+	+	=	0	1
Infected	+	-	+	-	+	1	1
Infected	+	-	+	-	-	1	0
Infected	+	NR	+	+	+	0	1
Infected	-	+	+	+	+	1	0

Table 12b: N. gonorrhoeae Infected Status for Patient-collected Vaginal Swab Specimens (continued)

Patient Infected Status	NAAT 1		NAAT 2		AGC Assay	Symptom Status	
	PVS	FU	PVS	FU		Symptomatic	Asymptomatic
Non-infected	+	-	-	-	-	2	0
Non-infected	-	-	+	+	+	1	0
Non-infected	-	-	+	-	-	2	2
Non-infected	-	-	-	+	-	0	2
Non-infected	-	-	-	-	+	0	1
Non-infected	-	-	-	-	-	961	1,064
Non-infected	-	-	-	-	=	1	1
Non-infected	-	-	-	NR	-	1	0
Non-infected	-	-	NR	-	-	12	10
Non-infected	-	-	NR	NR	-	0	1
Non-infected	-	NR	-	-	-	37	25
Non-infected	NR	-	-	-	-	3	6
Non-infected	NR	NR	-	-	-	42	25

AGC Assay = Aptima Neisseria gonorrhoeae Assay; **PVS** = patient-collected vaginal swab; **FU** = female urine; **NR** = no result.
Note: The equal symbol (=) represents a final equivocal result.

RLU Distribution of Aptima GC Assay Controls

The distribution of the RLU values for the Aptima GC Assay controls is presented in Table 13 from all valid Panther System runs performed during the clinical study that included patient-collected vaginal swabs, and female and male urine specimens.

Table 13: RLU Distribution of Aptima GC Assay Negative and Positive Controls

Control	Statistic	Total RLU (x1,000)
Positive Control, GC / Negative Control, CT	N	161
	Minimum	2,416
	Median	5,543.0
	Maximum	6,477
	CV%	14.62
Positive Control, CT / Negative Control, GC	N	161
	Minimum	2
	Median	4.0
	Maximum	40
	CV%	93.85

CV% = percent coefficient of variation; **RLU** = relative light unit.
Note: The RLU value reported by the software was the basis for analysis. The reported RLU value is the total measured RLU divided by 1,000 with the digits after the decimal point truncated.

Clinical Specimen Agreement

The Aptima GC Assay was first launched on semi-automated DTS Systems and then the Tigris DTS System. In 2010, indications were expanded to use the Aptima GC Assay on the Panther System. The Panther System is an alternative, smaller instrument platform to the Tigris DTS System. Both systems are intended to fully automated amplified nucleic acid testing of diagnostics assays. Select assay performance testing completed on the semi-automated DTS Systems and Tigris DTS System were leveraged to support assay performance on the Panther System.

Sensitivity, specificity, and predictive values of the Aptima GC Assay were established using the DTS System. 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 Solution 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 Hologic. 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 Solution Pap Specimens

Female and male subjects attending STI, 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 Solution Pap specimens. The specimens were transferred directly to Hologic for testing. At Hologic, 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 Solution 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 Solution 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 Solution 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 14 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 Solution 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 5b for PreservCyt Solution 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 Solution Pap specimens would be expected to be similar given the agreement findings.

Table 14: 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)
Sym	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 Solution	Female	34	28	0	0	6	100 (87.7–100)	100 (54.1–100)	100 (89.7–100)
Asym	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 Solution	Female	17	12	0	0	5	100 (73.5–100)	100 (47.8–100)	100 (80.5–100)

"+" denotes a positive result; "-" a negative result; **Sym** = symptomatic; **Asym** = asymptomatic; **CI** = confidence interval.

*Endocervical and vaginal swab samples combined.

[1]One disagreement in vaginal swab.

Table 14: Clinical Specimen Agreement Study: Positive, Negative, and Overall Agreements by Symptom Status (continued)

Symptom	Specimen	Gender	n	DTS+ Tigris+	DTS+ Tigris-	DTS- Tigris+	DTS- Tigris-	Positive % Agreement (95% CI)	Negative % Agreement (95% CI)	Overall % Agreement (95% CI)
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 Solution	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; **Sym** = symptomatic; **Asym** = asymptomatic; **CI** = confidence interval.

*Endocervical and vaginal swab samples combined.

[1]One disagreement in vaginal swab.

GC rRNA Spiked Clinical Panel Study

The GC rRNA spiked clinical panel study evaluated agreement between the two systems using six Hologic 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 Solution Pap specimens that had negative Aptima GC results on the DTS Systems when tested at Hologic. 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 15 shows the percent agreement for each level of rRNA in the endocervical swab, vaginal swab, urethral swab, male urine, female urine, and PreservCyt Solution 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 15 are the overall percent agreements of the clinical panel study between the Tigris DTS System and DTS Systems.

Table 15: 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	

Table 15: 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)
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	
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	

CI = confidence interval. *Not tested on both systems due to insufficient sample volume.

Analytical Performance

Analytical Sensitivity (DTS System)

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 Pap specimens). Because the reagents for the Aptima GC Assay are identical between DTS and Panther Systems, data generated on the DTS System supports the performance of the assay on Panther System.

Analytical Sensitivity (Panther System)

Analytical sensitivity of the Aptima GC Assay was tested using four representative sample matrices. These were urine, PreservCyt Solution Pap specimens, vaginal swab specimens, and STM. Panels were made by spiking GC rRNA into pools of these matrices at 12.5 CFU/mL and 125 CFU/mL (25 fg/assay and 250 fg/assay). These panels were tested on three Panther instruments using two lots of reagents in replicates of 60. Positive agreement with the expected result was calculated. Agreement to expected results was 100% (95% CI 95.7–100%) for all urine panels, for all PreservCyt Solution Pap specimen panels, for all vaginal swab specimen panels, and for all STM panels. The analytical sensitivity for the Aptima GC Assay is 125 CFU/mL.

Analytical Specificity

A total of 155 culture isolates were evaluated using the Aptima GC Assay. These isolates included 87 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, C. trachomatis*, and the viruses were tested at 1.0×10^6 cells/assay in KOVA-Trol® media/Urine Transport Media (UTM) and 60 organisms were tested in STM. 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. *C. trachomatis* was tested at 1.0×10^4 IFU/mL. The viruses were tested as follows: (a) herpes simplex virus I: 2.5×10^4 $TCID_{50}$ (Tissue Culture Infection Dose50)/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 16.

Table 16: Analytical Specificity

Organism	Organism	Organism
Achromobacter xerosis	*Escherichia coli*	*Neisseria sicca (3)*
Acinetobacter calcoaceticus	*Flavobacterium meningosepticum*	*Neisseria subflava (14)*
Acinetobacter lwoffi	*Fusobacterium nucleatum*	*Neisseria perflava*
Actinomyces israelii	*Gardnerella vaginalis*	*Neisseria polysaccharea*
Actinomyces pyogenes	*Gemella haemolysans*	*Paracoccus denitrificans*
Aerococcus viridans	*Haemophilus ducreyi*	*Peptostreptococcus anaerobius*
Aeromonas hydrophila	*Haemophilus influenzae*	*Peptostreptococcus productus*
Agrobacterium radiobacter	Herpes simplex virus I	*Plesiomonas shigelloides*
Alcaligenes faecalis	Herpes simplex virus II	*Propionibacterium acnes*
Bacillus subtilis	Human papillomavirus 16	*Proteus mirabilis*

Table 16: Analytical Specificity (continued)

Bacteriodes fragilis	*Kingella dentrificans*	*Proteus vulgaris*
Bacteriodes ureolyticus	Kingella kingae	*Providencia stuartii*
Bifidobacterium adolescentis	*Klebsiella oxytoca*	*Pseudomonas aeruginosa*
Bifidobacterium brevi	*Klebsiella pneumoniae*	*Pseudomonas fluorescens*
Branhamella catarrhalis	*Lactobacillus acidophilus*	*Pseudomonas putida*
Brevibacterium linens	*Lactobacillus brevis*	*Rahnella aquatilis*
Campylobacter jejuni	*Lactobacillus jensonii*	*Rhodospirillum rubrum*
Candida albicans	*Lactobacillus lactis*	*Saccharomyces cerevisiae*
Candida glabrata	*Legionella pneumophila (2)*	*Salmonella minnesota*
Candida parapsilosis	*Leuconostoc paramensenteroides*	*Salmonella typhimurium*
Candida tropicalis	*Listeria monocytogenes*	*Serratia marcescens*
Chlamydia pneumoniae	*Micrococcus luteus*	*Staphylococcus saprophyticus*
Chlamydia psittaci (2)	*Moraxella lacunata*	*Staphylococcus aureus*
Chlamydia trachomatis	*Moraxella osloensis*	*Staphylococcus epidermidis*
Chromobacterium violaceum	*Morganella morganii*	*Streptococcus agalactiae*
Citrobacter freundii	*Mycobacterium smegmatis*	*Streptococcus bovis*
Clostridium perfringens	*Mycoplasma genitalium*	*Streptococcus mitis*
Corynebacterium genitalium	*Mycoplasma hominis*	*Streptococcus mutans*
Corynebacterium xerosis	*N. meningitidis* Serogroup A	*Streptococcus pneumoniae*
Cryptococcus neoformans	*N. meningitidis* Serogroup B	*Streptococcus pyogenes*
Cytomegalovirus	*N. meningitidis* Serogroup C (4)	*Streptococcus salivarius*
Deinococcus radiodurans	*N. meningitidis* Serogroup D	*Streptococcus sanguis*
Derxia gummosa	*N. meningitidis Serogroup Y*	*Streptomyces griseinus*
Eikenella corrodens	*N. meningitidis* Serogroup W135	*Trichomonas vaginalis*
Enterobacter aerogenes	*Neisseria cinerea (4)*	*Ureaplasma urealyticum*
Enterobacter cloacae	*Neisseria dentrificans*	*Vibrio parahaemolyticus*
Entercoccus avium	*Neisseria elongata (3)*	*Yersinia enterocolitica*
Entercoccus faecalis	*Neisseria flava*	
Entercoccus faecium	*Neisseria flavescens (2)*	
Erwinia herbicola	*Neisseria lactamica (9)*	
Erysipelothrix rhusiopathiae	*Neisseria mucosa (3)*	

(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 Solution 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 x 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.

Reproducibility Study

Aptima GC Assay reproducibility was evaluated at two external US laboratories and at Hologic using the Panther System. Testing was performed over six days using two lots of assay reagents and a total of six operators (two at each site). Reproducibility panel members were created using clinical urine specimens. The GC positive panel members were created using naturally infected individual or pooled GC positive samples that were diluted with volume from pooled negative samples to result in panel members with expected mean targeted RLU ranges (low positive or positive).

Table 17 presents, for each panel member, RLU data in terms of mean, standard deviation (SD), and coefficient of variation (CV) between sites, between operators, between lots, between runs, within runs, and overall. Percent agreement with expected results is also shown. Samples with valid results were included in the analyses.

Table 17: Panther System Reproducibility Data

Panel Member	Agreed/N	Agrmt (%)	Mean RLU (x1000)	Between Sites		Between Operators		Between Lots		Between Runs		Within Runs		Total	
				SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
Negative	108/108	100	2.1	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.2	11.9	0.2	12.0
Low Positive	105/105[1]	100	926.1	81.2	8.8	0.0	0.0	98.4	10.6	86.2	9.3	361.5	39.0	392.9	42.4
Positive	107/107[2]	100	6196.9	247.5	4.0	23.7	0.4	315.1	5.1	136.5	2.2	187.4	3.0	463.5	7.5

Agrmt = agreement; **CV (%)** = coefficient of variation; **N** = number of panel members; **SD** = standard deviation; **RLU** = relative light unit.

[1]There were 3 invalid results excluded from the analysis.

[2]There was 1 invalid result excluded from the analysis.

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

Carryover Study for the Panther System

A multi-run analytical study was conducted using spiked panels on three Panther Systems. Carryover was assessed using approximately 20% high titer GC samples dispersed between negative samples. The runs included clusters of high positive samples with clusters of negative samples as well as single high positives dispersed within the run. High titer samples were made using GC rRNA spiked into STM to give a final concentration of 5 x 10^5 fg rRNA/reaction (rRNA equivalent of 2.5 x 10^5 CFU/mL). Testing was carried out using 5 runs on three Panther Systems with a total of 2,939 negative samples. The overall carryover rate was 0.07% with a 95% confidence interval of 0.02–0.25%.

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