Aptima® Neisseria gonorrhoeae Assay

For *in vitro* diagnostic use.

Rx 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 urine from symptomatic and asymptomatic male individuals.

Summary and Explanation of the Test

GC infections are one of the most common sexually transmitted infections worldwide. In the United States, an estimated 1,568,000 new *GC* infections occur each year (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. Untreated infections in men can cause urethritis and epididymitis (6). A small percentage of persons with gonococcal infections may develop Disseminated Gonococcal Infection (DGI) (8).

Conventional diagnosis of GC infection requires isolation of the organism on selective media or the observation of diplococci in Gram stained smears (6). 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, 7). Commonly used non-culture methods for GC detection include direct DNA probe tests and NAATs.

The Aptima GC assay is a 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 the target capture, TMA, and HPA technologies used in the Aptima GC assay (3, 4).

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.

Principles of the Procedure

The Aptima GC assay combines the technologies of target capture, TMA, and HPA.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the Aptima GC assay is performed in the laboratory, the target rRNA molecule is isolated from the specimens using 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 hybridization 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-based hybridization protection assay. 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 and precautions, refer to the *Panther/Panther Fusion System Operator's Manual*.

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

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.

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

Laboratory Related

G. Use only supplied or specified disposable laboratory ware.

H. 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.

I. **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.

J. 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.

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

L. 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

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. 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.

O. 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.

P. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers from different patients do not contact one another during specimen handling in the laboratory. Change gloves if they come in contact with a specimen.

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

R. 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.

S. Upon piercing, liquid can discharge from Aptima transport tube caps under certain conditions. Follow instructions in the *Panther System Test Procedure* to prevent this occurrence.

T. Do not use material that may contain guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

Assay Related

U. 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* and *Panther System Test Procedure* for more information.

V. Use Universal Precautions when handling controls.

W. Avoid microbial and ribonuclease contamination of reagents.

X. Do not use a kit or control after its expiration date.

Y. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Aptima Controls and assay fluids may be interchanged.

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

AA. Some reagents of this kit are labeled with risk and safety symbols.

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 on www.hologic.com/package-inserts.*

US Hazard Information	
—	**Amplification Reagent** *HEPES 25 - 30%* — Harmful to aquatic life with long lasting effects. Harmful to aquatic life
	**Selection Reagent** *Boric Acid 1 - 5%* **WARNING** H315 - Causes skin irritation P264 - Wash face, hands and any exposed skin thoroughly after handling P280 - Wear protective gloves/protective clothing/eye protection/face protection
—	**Probe Reagent** *Succinic Acid 10 - 15%* *Lithium Hydroxide, Monohydrate 10 - 15%* — Harmful to aquatic life with long lasting effects. Harmful to aquatic life
—	**Target Capture Reagent** *HEPES 5 - 10%* *EDTA 1 - 5%* *Lithium Hydroxide, Monohydrate 1 - 5%* — Harmful to aquatic life with long lasting effects. Harmful to aquatic life

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	60 days
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 Amplification Reagent, Enzyme 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. Reagents stored on-board the Panther system have 72 hours of on-board stability.

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

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.*

Note: *Do not use medium that may contain guanidinium thiocyanate or any guanidine-containing material.*

The Aptima GC assay is designed to detect the presence of GC in male urine specimens. Performance with specimens other than those collected with the Aptima Urine Collection Kit for male urine has not been evaluated.

A. Specimen Collection

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

B. Specimen Transport and Storage Before Testing

1. Male 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 (see *Specimen Stability Studies*).

C. Specimen Storage After Testing

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen transport tubes should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable caps and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 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..*	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.*	5 x 1.7 mL

Materials Required but Available Separately

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

	Cat. No.
Panther System	303095
Panther System Continuous Fluid and Waste (Panther Plus)	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 Urine Specimen Collection Kit for Male and Female Urine Specimens	301040
Aptima Urine Specimen Transport Tubes	105575
Bleach, 5% to 8.25% (0.7M to 1.16M) 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	—
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens* **used for lab contamination monitoring*	301041

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

B. Reagent Reconstitution/Preparation of a New Kit

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

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the reconstitution solution bottle opening (Figure 1, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
   g. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (Figure 1, Step 4).

h. 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.

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

j. Recap the reconstitution solution bottle. Record operator initials and the reconstitution date on the label (Figure 1, Step 7).

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

Option: Additional mixing of the Amplification, Enzyme and Probe Reagents using a tube rocker is allowed by placing the recapped plastic bottle on a tube rocker set at 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.*

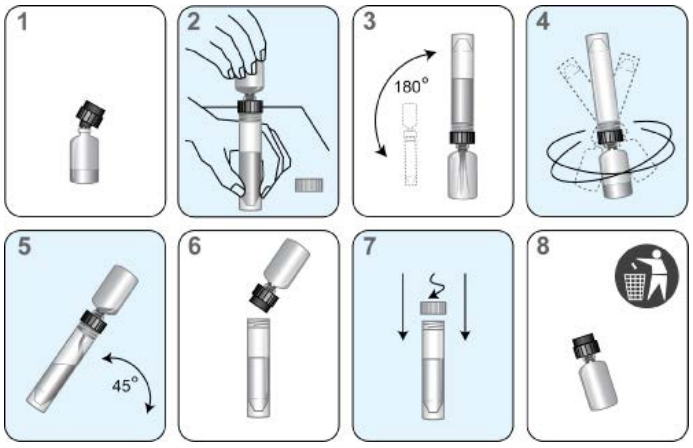

Figure 1. Panther System Reconstitution Process

2. Prepare 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 moderate speed and tilt for a minimum of 25 minutes to ensure 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.
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. Specimen Handling

1. Allow the controls and specimens to reach room temperature prior to processing.
2. Do not vortex specimens.
3. Visually confirm that each specimen tube has a final volume of urine between the black fill lines of a urine specimen transport tube.
4. 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 4a–c may result in liquid discharge from the specimen tube cap.*

Note: *Up to 4 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 4 aliquots from the specimen tube can lead to processing errors.*

E. 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 Aptima assay software on the Panther system, 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 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:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the 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.

For test interpretation, 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 Aptima GC assay software. 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.

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

* If the RLU measured on the Panther system is between zero and 999 RLU, a result of "0" is reported in the "Total RLU (000s)" column in the run report. Measured RLU values less than 690 are reported as invalid. RLU values between 690 and 999 are reported as valid.

[1] 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. See *Optional Materials*. Correct preparation of specimens is confirmed visually by the final volume of urine in between the black fill lines of a urine specimen transport tube.

The Controls must produce the following test results:

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

* If the RLU measured on the Panther system is between 0 and 999, a result of "0" is reported in the "Total RLU (000s)" column in the run report. Measured RLU values less than 690 are reported as invalid. RLU values between 690 and 999 are reported as valid.

1. Each laboratory should implement appropriate control procedures to satisfy the requirements of CLIA regulations.
2. Negative controls may not be effective in monitoring random carryover. See *Panther System 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. Urine 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 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.

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 specimen collection variables have not been assessed for their impact on the detection of GC.

C. The Aptima GC assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications.

D. This assay has been tested using male urine specimens only. Performance with specimens other than those specified under *Specimen Collection and Storage* has not been evaluated.

E. 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.

F. Therapeutic failure or success cannot be determined with the Aptima GC assay since nucleic acid may persist following appropriate antimicrobial therapy.

G. Results from the Aptima GC assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

H. 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.

I. 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.

J. For the male urine specimen clinical study, 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.

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

L. The performance of the Panther system has not been evaluated at altitudes above 6561 feet (2000 m).

M. Customers must independently validate an LIS transfer process.

Panther System Expected Values

Estimates of *N. gonorrhoeae* 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 *N. gonorrhoeae* for male urine, as determined by the Aptima GC assay on the Panther system, is shown in Table 1 for the multicenter study, by clinical site and overall.

Table 1: Positivity of N. gonorrhoeae as Determined by the Aptima GC Assay in Male Urine Specimens by Clinical Site

	Positivity % (# positive/# tested with valid non-equivocal results)
Site	**MU**
1	21.7 (38/175)
2	0.8 (3/373)
3	0 (0/61)
4	0 (0/13)
5	8.3 (34/409)
6	9.4 (29/307)
7	5.3 (12/225)
8	0 (0/32)
9	0 (0/218)
10	11.0 (10/91)
11	0 (0/54)
All	6.4 (126/1958)

MU = male urine.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive (PPV) and negative (NPV) predictive values of the Aptima GC assay across different hypothetical prevalence rates are shown for male urine in Table 2. 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 3).

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

Specimen Type		Hypothetical Prevalence						
		1%	**2%**	**5%**	**10%**	**15%**	**20%**	**25%**
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

MU = male urine, NPV = negative predictive value, PPV = positive predictive value.

Panther System Clinical Performance

Clinical Study

A prospective, multi-center clinical study was conducted to establish the clinical performance characteristics of the Aptima GC assay on the Panther system. Specimens were collected from 2085 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 twenty-six (126) enrolled subjects were not evaluable (12 were withdrawn and 114 did not have at least one specimen with a valid non-excluded Aptima result and a conclusive infected status). The average age among evaluable study subjects was 35.6 years (range = 14 to 84 years). Symptoms were reported in 42.1% (825/1959) of the evaluable subjects.

One 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. Male urine specimens were tested with up to 3 FDA-cleared NAATs to establish the patient infected status (PIS).

Specimens were categorized as infected if a positive result occurred in at least two of the comparator NAATs, and as not infected if at least 2 of the comparator results were negative; the third (tie-breaker) comparator was only required if the first 2 comparator results were discordant. Specimens that could not be categorized, due to missing results from the comparator assays, were excluded from the performance analyses. Performance of the Aptima GC assay was estimated relative to the PIS.

Of the specimens collected, 2042 were processed in valid Aptima GC assay runs, including 85 (4.2%) that had to be retested due to invalid results. Overall, 2030 (99.4%) had final valid results, and 12 (0.6%) had final invalid results and were excluded from the analyses. A total of 1958 male urine specimens from evaluable subjects were included in the analyses comparing Aptima GC assay results to the PIS: one specimen with final GC equivocal results was excluded from the performance analyses.

Performance Results

Table 3 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 male urine specimens by symptom status and overall.

Table 3: Performance Characteristics of the Aptima GC Assay in Male 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]
MU	All	1958	125	1	1830	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	825	105	1	717	2	13.0	98.1 (93.4, 99.5)	99.9 (99.2, 100)	99.1 (95.1, 100)	99.7 (99.0, 100)
	Asym	1133	20	0	1113	0	1.8	100 (83.9, 100)	100 (99.7, 100)	100 (84.4, 100)	100 (99.7, 100)

Asym = asymptomatic, CI = confidence interval, FN = false negative, FP = false positive, MU = male urine, Prev = prevalence, Sym = symptomatic, TN = true negative, TP = true positive.

[1]This specimen also tested positive using an alternative *N. gonorrhoeae* NAAT assay with the following results (# positive results / # samples tested): 1/1.

[2]These specimens were also tested by an alternative *N. gonorrhoeae* NAAT assay with the following results: 1 specimen was negative by the alternative NAAT and 1 specimen was positive by the alternative NAAT.

[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 4 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 PIS) by collection site. Prevalence varied across collection sites, as expected.

Table 4: 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]
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)

CI = confidence interval, FN = false negative, FP = false positive, MU = male urine, NC = not calculable, Prev = prevalence, TN = true negative, TP = true positive.

[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.

Note: One evaluable male urine specimen with a final equivocal GC assay result was excluded from the performance analysis.

Neisseria gonorrhoeae Infected Status Tables

The frequency of test outcomes from reference NAAT and investigational Panther system testing is summarized in Table 5a.

Table 5a: N. gonorrhoeae Infected Status for Male Urine Specimens

Specimen Type	Patient Infected Status	NAAT 1	NAAT 2	NAAT 3	AGC Assay	Symptom Status	
						Sym	Asym
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	1079
	Non-infected	-	-	N/A	=	0	1
	Non-infected	-	NR	-	-	1	0
	Non-infected	NR	-	-	-	26	32

Asym = asymptomatic, AGC Assay = Aptima Neisseria gonorrhoeae Assay, MU = male urine, N/A = not applicable, NR = no result, Sym = symptomatic.
Note: The equal symbol (=) represents a final equivocal result.

RLU Distribution of Aptima GC Assay Controls

The distribution of the RLUs for the Aptima GC assay controls is presented in Table 6 from all valid Panther system runs performed during the clinical study.

Table 6: RLU Distribution of Aptima GC Assay Negative and Positive Controls

Control	Statistic	Total RLU (x1000)
Positive Control, GC / Negative Control, CT	N	131
	Minimum	2416
	Median	5601.0
	Maximum	6362
	CV%	13.56
Positive Control, CT / Negative Control, GC	N	131
	Minimum	2
	Median	4.0
	Maximum	40
	CV%	92.80

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 1000 with the digits after the decimal point truncated.

Panther System Analytical Performance

Analytical Sensitivity

Analytical sensitivity of the Aptima GC assay was tested using urine specimens. 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. The analytical sensitivity for the Aptima GC assay is 125 CFU/mL.

The limit of detection (LoD) was further tested and confirmed with sensitivity panels prepared using two strains of GC organisms spiked into pooled negative urine. Testing evaluated one antibiotic susceptible strain; *Neisseria gonorrhoeae* ATCC 49226 (GP1803), and one antibiotic resistant stain; *Neisseria gonorrhoeae* WHO X/NCTC 13820 (GP2730). Sensitivity panels were tested on three Panther instruments with two reagent lots. At least 20 replicates were run for each concentration for each reagent lot for each strain. LoD, defined as the target concentration that can be detected in 95% of the replicates tested for urine specimens is 0.04933 CFU/mL for ATCC 49226 and 0.03986 CFU/mL for stain X/NCTC 13820.

Analytical Specificity

A total of 155 culture isolates were evaluated using the Aptima GC assay on the DTS® System or Panther system. 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 x 10^6 cells/assay. *C. psittaci* VR601 was tested at 8.0 x 10^4 cells/assay and *C. psittaci* VR125 was tested at 1.0 x 10^5 cells/assay. *C. pneumoniae* was tested at 4 x 10^3 cells/assay and *U. urealyticum* was tested at 6.7 x 10^6 cells/assay. *C. trachomatis* was tested at 1.0 x 10^4 IFU/mL. The viruses were tested as follows: (a) herpes simplex virus I: 2.5 x 10^4 $TCID_{50}$/assay, (b) herpes simplex virus II: 6.0 x 10^4 $TCID_{50}$/assay, (c) human papillomavirus 16: 2.9 x 10^6 DNA copies/assay and (d) cytomegalovirus: 4.8 x 10^5 cells/assay.

The list of organisms tested is shown in Table 7.

Table 7: 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 papilloma virus 16	*Proteus mirabilis*
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*

Table 7: Analytical Specificity (continued)

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.

Note: *For a nucleic acid amplification assay, analytical specificity with respect to individual organisms is largely determined by the chemistry of the assay (e.g. oligonucleotide sequences), rather than by the platform. Because the reagents for the Aptima GC Assay are identical between the Panther system and the DTS systems, analytical specificity for the DTS system is shown in Table 7.*

Interfering Substances

For a nucleic acid amplification assay, analytical specificity with respect to potentially interfering substances is largely determined by the chemistry of the assay (e.g. oligonucleotide sequences) rather than by the platform. Because the reagents for the Aptima GC assay are identical between DTS and Panther systems, data generated on the DTS system supports the performance of the assay on Panther system.

The following interfering substances were individually spiked into 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.

Mucin and seminal fluid were tested on the Panther system by spiking into pooled negative urine negative in the presence or absence of *Neisseria gonorrhoeae* ATCC 49226. Mucin (at 0.1% vol/vol) and seminal fluid (at 1% vol/vol) did not interfere with the assay.

Whole blood was used to establish the degree of blood interference on the Panther system with respect to this potential interferant. Fresh blood was added to clinical pools of urine specimens and then tested for potential assay interference in the presence and absence of GC target. The estimated rRNA equivalent of 125 GC CFU/mL (250 fg/assay) was used as the target concentration as this represents the analytical sensitivity of the assay. Specimens were tested on the Panther system. All samples containing target nucleic acid were positive when tested at a level of 30% (vol/vol) blood in urine specimens. All samples that did not contain target were correctly identified as negative. Blood, added to urine specimens at levels much higher than could be expected with normal specimen collection, did not interfere with results on the Panther system.

Carryover Studies 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 5×10^5 fg rRNA/assay (rRNA equivalent of 2.5×10^5 CFU/mL). Testing was carried out using 5 runs on three Panther systems with a total of 2939 negative samples. The overall carryover rate was 0% with a 95% confidence interval of 0.0–0.1%. A total of 17 negative samples from the high titer runs were reported as invalid and were excluded from the calculation.

Reproducibility Study

Aptima GC assay reproducibility was evaluated on the Panther system at two external US laboratories and at Hologic. Testing was performed using two lots of assay reagents and a total of six operators (two at each site). At each site, testing was performed over at least six days.

Reproducibility panel members were created using clinical urine specimens in Urine Transport Medium (UTM). The GC positive panel members were created by diluting GC positive clinical urine specimens with volume from pooled GC negative clinical urine specimens to achieve the appropriate targeted RLU results (low positive or positive). The negative panel member was created using pooled GC negative clinical urine specimens.

The agreement with expected results was 100% for all panel members.

Table 8 shows the signal variability of assay RLU results for each panel member between sites, between operators, between lots, between runs, within runs, and overall (Total). Only samples with valid results were included in the analyses.

Table 8: Reproducibility Study Data: Signal Variability by Panel Member

Panel Member	Target RLU (x1000)	N	Mean RLU (x1000)	Between Sites		Between Operators		Between Lots		Between Runs		Within Runs		Total	
				SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
Negative	<50	108	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	100 to <2000	105[1]	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	2000 to <12000	107[1]	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

CV = coefficient of variation, RLU = relative light unit, SD = standard deviation.

Notes: The RLU value reported by the software is the total measured RLU divided by 1000 with the digits after the decimal point truncated. Variability from some factors may be numerically negative. In these cases, SD and CV are shown as 0.0.

[1]Invalid results were excluded from the analyses for low positive (n=3) and positive (n=1) panel members.

Within-lab Precision Study

Precision was measured using positive panel members consisting of Neisseria gonorrhea cells at three different concentrations approximately: 3X LoD, (Low Positive), >3X and <5X LoD, (Moderate Positive), and >10X LoD (High positive). Cells were spiked into pooled negative urine matrix (Urine mixed 1:1 with Urine Transport Media). The negative panel member consisted of unspiked negative urine matrix. Testing was conducted over the course of at least 20 non-consecutive days using two lots of reagents on three Panther systems by three operators performing at least two daily runs. Agreement to expected results was 100% for all four panel members. The precision of the signal for each panel is shown in Table 9.

Table 9: Signal Variability by Panel Member

Panel Member	[GC] (CFU/mL)	N	Mean RLU (x1000)	Agmt	Between Lot		Between Instrument		Between Operator		Between Day		Between Run		Within Run		Totals	
					SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
LPos	0.1431	162	520.1	100%	65.05	12.51	38.39	7.38	0.00	0.00	14.84	2.85	20.11	3.87	62.34	11.99	101.07	19.43
MPos	0.2417	162	809.4	100%	90.32	11.16	52.74	6.52	7.71	0.95	15.00	1.85	24.09	2.98	84.36	10.42	137.55	16.99
HPos	0.5426	162	1529.4	100%	182.18	11.91	78.41	5.13	19.09	1.25	51.45	3.36	0.00	0.00	111.09	7.26	233.86	15.29
Neg	0.000	162	4.2	100%	1.19	28.36	0.87	20.80	0.51	12.28	0.00	0.00	0.00	0.00	3.52	84.17	3.85	92.04

Agmt = agreement to expected result, **[GC]** = *N. Gonorrhoeae* concentration, **RLU** = relative light units, **SD** = standard deviation, **CV (%)** = coefficient of variation, **HNeg** = high negative, **LPos** = low positive, **MPos** = moderate positive, **HPos** = high positive, **Neg** = negative.

Note: The analysis was performed using SAS PROC MIXED, which applies a lower boundary of 0 to all variance components in the model by default. If a variance component is 0, then SD=0 and CV=0%.

Specimen Stability Studies

The following specimen stability was evaluated using the DTS system and/or the Tigris® DTS System.

A. Urine Specimens

Data to support the recommended shipping and storage conditions for urine samples were generated with negative urine samples. The urine samples were spiked with GC at a final concentration of 100 CFU per reaction. The samples were held at 30°C for 24 hours prior to being added to the UTM. The UTM samples then were held at 4°C and 30°C and tested in triplicate at days 1, 14, 32 and 35. All replicates were positive for GC with UTM samples held at 4°C and 30°C.

B. Frozen Specimen Stability Study

The recommended frozen storage conditions for urine specimens in transport media is between –20°C to –70°C for up to 12 months after collection. Supporting data were generated using 90 negative specimens. Of these, 30 specimens were spiked with GC at 50 CFU per reaction; 30 specimens were spiked with GC at 5 CFU per reaction; and 30 specimens were not spiked. The specimens in transport media were stored frozen within 7 days of collection and tested at days 200 and 400. Specimens met the acceptance criteria of 95% agreement with expected results.

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AW-28505-001 Rev. 001
2024-02