

Laboratory Procedure Manual

Analyte: Mycoplasma genitalium

Matrix: Urine

Method: Hologic APTIMA Mycoplasma genitalium Assay

Method No.:

Revised: September 12, 2019

As performed by: Division STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD, TB Prevention
Centers for Disease Control and Prevention

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Important Information for Users

The Division of STD Prevention periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
MGEA_K_R MGEN_K_R	URXMYGE	Mycoplasma genitalium, urine

1) SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Mycoplasma genitalium is a sexually-transmitted fastidious bacterium that is difficult to culture. The APTIMA *Mycoplasma genitalium* (*M. genitalium*) Assay is an *in vitro* nucleic acid amplification test (NAAT) for the qualitative detection of ribosomal RNA from *M. genitalium*. This assay combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and hybridization protection assay (HPA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the rRNA targets and protect them from degradation during storage. When the APTIMA *M. genitalium* Assay is performed in the laboratory, the target 16s rRNA molecule, if present, is isolated from specimens by the use of a specific capture oligomer and magnetic microparticles in a method called target capture. The capture oligomer contains sequences complementary to 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 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 molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the rRNA is 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 small ribosomal subunit from *M. genitalium* via DNA and RNA intermediates and generates RNA amplicon molecules. Detection of the rRNA amplification product sequences is achieved using nucleic acid hybridization (HPA). A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with different 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).

2) SAFETY PRECAUTIONS

A. Reagent Toxicity

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

Work surfaces, pipettes, and other equipment must be regularly decontaminated with a 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Thoroughly clean and disinfect all work surfaces.

B. Microbiological Hazards

Specimens may be infectious. Use Universal Precautions when performing this assay.

- If a spill occurs, immediately disinfect following appropriate site procedures.
- Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over any open container. Change gloves if they come in contact with a specimen.

C. Protective Equipment

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

D. Training

Only personnel adequately trained in the use of the Hologic PANTHER system and APTIMA *M. genitalium* assay and in handling potentially infectious materials should perform this procedure.

E. Disposal of Wastes

Use only supplied or specified disposable laboratory ware.

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

3) COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- A. Specimens received from NHANES studies are labeled by the specimen ID and barcoded. Specimens tested in this laboratory with this procedure are derived from participants consented and enrolled in CDC IRB approved investigational studies.
- B. After the data is calculated, results are approved by the reviewing supervisor for release. All results are entered onto the specific study data file.

4) SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. Specimen Collection

Self-collected first-catch male and female urine specimens can be tested with the APTIMA *M. genitalium* assay.

B. Specimen Handling

Before urine specimens can be tested, urine must be transferred to an APTIMA urine transport tube in accordance with the instructions in the urine collection kit package insert. Do not vortex specimens. After urine has been added, the liquid level in the urine transport tube must fall between the two black indicator lines on the tube label.

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.

Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they contact specimen.

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

C. Storage

After collection, urine specimens in the primary collection container are stored at 2°C to 30°C for up to 24 hours before urine is transferred to the transport tube. Processed urine in the transport tube is stored at 2°C to 30°C for up to 30 days (after transfer). When longer storage is needed, processed urine in the transport tube is stored at -20°C or -70°C for up to an additional 90 days (after transfer).

D. Specimen Rejection

Specimen must be rejected if urine levels do not fall in between black indicator lines on the urine transport tube.

5) PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6) EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrumentation

1. Panther system
2. Circulating water bath

B. Materials

1. APTIMA *Mycoplasma genitalium* Assay Kit
2. APTIMA *Mycoplasma genitalium* Calibrators Kit
3. Tips, 1000 µL conductive, liquid sensing (Tecan)
4. APTIMA Urine Specimen Collection Kit
5. APTIMA Assay Fluids Kit
6. APTIMA Auto Detect Kit
7. APTIMA Penetrable Caps

8. Reagent replacement caps for 100-test kits
9. Multi-tube units (MTUs)
10. PANTHER System Run Kit
11. Disposable powderless gloves
12. Household bleach (sodium hypochlorite solution)
13. Hologic Bleach Enhancer for Cleaning

C. Reagent Preparation

- 1) Enzyme Reagent, Amplification Reagent, and Probe Reagent preparation:
 - a. Combine the lyophilized reagent with the appropriate reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
 - b. Remove the lyophilized reagents (2°C to 8°C) and corresponding reconstitution solutions (15°C to 30°C) from storage.
 - c. Before attaching the reconstitution collar, ensure that the reconstitution solution and lyophilized reagent have matching label colors.
 - d. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
 - e. Open the lyophilized reagent vial by removing the metallic seal and rubber stopper. Firmly insert the notched end of the reconstitution collar (black) into the vial
 - f. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
 - g. Place the reconstitution solution bottle on a stable surface (i.e., bench). Then invert the lyophilized reagent vial over the reconstitution solution bottle and firmly attach the collar to the reconstitution solution bottle.
 - h. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial.
 - i. Pick up the assembled bottles and gently swirl. Avoid creating foam while swirling the bottle.
 - j. Wait for the lyophilized reagent to go into solution. After the lyophilized reagent has gone into solution, gently swirl to mix, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming. Slowly tilt the assembled

bottles again to allow all the solution to drain back into reconstitution solution bottle.

- k. Carefully remove the reconstitution collar and glass vial.
- l. Recap the bottle. Record operator initials and reconstitution date on the label.
- m. Discard the reconstitution collar and glass vial.

Warning: Avoid creating foam when reconstituting reagents. Foam compromises the level sensing in the PANTHER system.

2) Target Capture Reagent (TCR) preparation:

- a. Remove the appropriate bottles of TCR (15°C to 30°C) and Internal Control Reagent (2°C to 8°C) from storage.
- b. Check the lot number on the TCR bottle and Internal Control Reagent bottle to make sure that the numbers match the lot number on the Master Lot Barcode Sheet.
- c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
- d. Open the bottle of Internal Control Reagent and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the Internal Control 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 Internal Control Reagent bottle and cap.

3) Selection Reagent preparation:

- a. Remove the Selection Reagent from storage (2°C to 30°C). Check the lot number on the Selection Reagent bottle to make sure the lot number matches the number on the Master Lot Barcode sheet.
- b. If the Selection Reagent is stored refrigerated let it come to room temperature before placing on the PANTHER system.
- c. 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.

4) Reagent Preparation for Previously Prepared Reagents:

- a. Remove the previously prepared reagents from storage (2°C to 8°C). Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.
- b. If reconstituted Probe Reagent contains precipitate at room temperature (15°C to 30°C), heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. Mix Probe Reagent by inversion. Avoid creating foam during inversion of reagents. After this heat step, the Probe Reagent may be used even if residual precipitate remains.
- c. Invert the Amplification, Enzyme, and Probe Reagents to mix thoroughly prior to loading on the system. Avoid creating excessive foam during inversion of reagents.
- d. Do not top off reagent bottles. The PANTHER system will recognize and reject bottles that have been topped off.

D. CALIBRATORS

1) Calibrator Preparation

- a. Remove the calibrators from storage (2°C to 8°C) and allow the calibrators to reach 15°C to 30°C prior to processing.

2) Calibrator Handling

- a. APTIMA Positive Calibrator for *M. genitalium* and APTIMA Negative Calibrator for *M. genitalium* are tested
- b. Each Calibrator can only be used once. Attempts to use the tubes more than once can lead to processing errors

E. CONTROLS

- 1) APTIMA *M. genitalium* Internal Control (IC) consists of non-infectious RNA transcript in buffered solution containing <5% detergent.
- 2) An IC is added to each sample (specimens and calibrators). During processing, IC acceptance criteria are automatically verified by the PANTHER system software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested.

7) CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

- A. To generate valid results, an assay calibration must be completed. Positive and negative calibrators are run in duplicate each time a reagent kit is loaded on the

PANTHER system. The PANTHER manual lists 24 hour calibrator stability, however, the APTIMA *Mycoplasma genitalium* assay calibration is valid for up to 48 hours. Software on the PANTHER system alerts the operator when a new calibrator set is required.

- B.** During processing, criteria for acceptance of the calibrator are automatically verified by the software on the PANTHER system. If two replicates are invalid for either the positive or negative calibrator, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared set of calibrators.
- C.** The APTIMA Positive Calibrator for *M. genitalium* and APTIMA Negative Calibrator for *M. genitalium* tubes can be loaded in any rack position or in any Sample Bay Lane on the PANTHER system. Specimen pipetting will begin when one of the following two conditions has been met:
 - 1) A pair of calibrators is currently being processed by the system.
 - 2) Valid results for the calibrators are registered on the system.
- D.** Once the calibrator tubes have been pipetted and are processing for the APTIMA *M. genitalium* assay reagent kit, specimens can be tested with the associated reconstituted kit for up to 48 hours unless:
 - 1) Calibrator results are invalid.
 - 2) The associated assay reagent kit is removed from the system.
 - 3) The associated assay reagent kit has exceeded stability limits.
- E.** Each calibrator tube can be used once. Attempts to use the tube more than once can lead to processing errors.

8) PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Operating the PANTHER System:

- 1) Before loading reagents and samples onto the PANTHER, perform instrument and laboratory check by recording room temperature (15°C-30°C) to make sure it falls within the acceptable ranges. Perform an external inspection of machine and check for any leaks.
- 2) Make sure workbenches have been cleaned. If not then clean with 2.5-3.5% sodium hypochlorite solution, let sit 1 minute and follow with distilled water rinse.

- Allow workbench to dry and cover with a plastic backed bench cover. Change gloves.
- 3) Prepare reagents as instructed if they are not already prepared. Remove gloves.
 - 4) Log on to PANTHER software using your login and password.
 - 5) Exit power save mode.
 - 6) Wearing clean gloves, load tips and multi-tube units (MTUs) if needed.
 - 7) Load universal fluids if necessary.
 - 8) Empty waste from waste drawer if needed. Change gloves.
 - 9) Ensure all maintenance is current; system will not operate if any maintenance is overdue.
 - 10) Prime if needed.
 - 11) Load assay reagents that are prepared as instructed above, making sure there are no bubbles in the reagents.
 - 12) Load samples and calibrators.
 - 13) Change gloves.
 - 14) Refer to the screen to make sure there are no pending messages or problems with specimens. If there are, identify what is needed and make corrections according to the operator manual or on-screen instructions.
 - 15) Return to system to load tips, MTUs, additional reagents and additional samples as needed. Change gloves between each task.
 - 16) Samples may be removed when pipetting is complete, and all samples are indicated in blue on the screen graphic. Calibrators are disposed of in a biohazard bag to prevent contamination. Make sure to keep tubes upright at all times.
 - 17) Sample racks and retainers should be placed in a bin of 2.5-3.5% sodium hypochlorite solution for at least 10 min, rinsed with tap water, and allowed to dry. Change gloves after this task.
 - 18) When run is complete, print the report "results by worklist".
 - 19) Reagent racks should be rinsed in the bin of 2.5-3.5% sodium hypochlorite solution and rinsed as were sample racks. Change gloves.
 - 20) If reagents are not all used, they may be left on the machine for the following day if a run is to be performed. Otherwise, remove them, re-cap with new caps and store in the refrigerator. Store TCR reagent in the reagent prep area at room temperature.

B. Calculations

1) Internal Control (IC) Cutoff Calculation

- a. The IC cutoff is determined from the IC signal from valid Negative Calibrator replicates.
- b. $IC\ Cutoff = 0.5 \times [mean\ IC\ Relative\ Light\ Unit\ (RLU)\ of\ the\ valid\ Negative\ Calibrator\ replicates]$

2) Analyte Cutoff Calculation

- a. The analyte cutoff is determined from the RLU signal from valid Negative Calibrator replicates and valid Positive Calibrator replicates.
- b. $Analyte\ Cutoff = [1 \times mean\ analyte\ RLU\ of\ valid\ Negative\ Calibrator\ replicates] + [0.035 \times mean\ analyte\ RLU\ of\ the\ valid\ Positive\ Calibrator\ replicates]$

3) Analyte Signal to Cutoff (S/CO) Calculation

- a. The analyte S/CO is determined from the analyte RLU of the test sample and the analyte cutoff for the run.
- b. $Analyte\ S/CO = test\ sample\ analyte\ RLU \div analyte\ cutoff$

C. Data Analysis and Interpretation of Results

Assay test results are automatically interpreted by the PANTHER system APTIMA *M. genitalium* Assay software. A test result may be negative, positive, or invalid as determined by the Internal Control (IC) Relative Light Unit (RLU) and Signal to Cutoff (S/CO) ratio for the Analyte in the detection step (Table 1). A test result may be invalid due to RLU values outside the normal expected ranges. Initial invalid test results should be retested. Report the first valid result.

Table 1: Test Interpretations based on Results

Result	Negative	Positive	Invalid
Criteria	<p>Analyte S/CO < 1.0</p> <p>IC ≥ IC Cutoff</p> <p>IC ≤ 1,200,000 RLU</p>	<p>Analyte S/CO ≥ 1.0</p> <p>IC ≤ 1,200,000 RLU</p> <p>Analyte ≤ 3,000,000 RLU</p>	<p>Analyte S/CO < 1.0 and IC < IC Cutoff</p> <p>Or</p> <p>IC > 1,200,000 RLU</p> <p>Or</p> <p>Analyte > 3,000,000 RLU</p>

9) REPORTABLE RANGE OF RESULTS

A positive, negative, or invalid are the reportable range of results.

10) QUALITY CONTROL (QC) PROCEDURE

A. Positive & Negative Controls

Controls must be run with each assay. The APTIMA Positive Calibrator and Negative Calibrator act as controls for the Target Capture, Amplification, and Detection steps of the assay. Software on the PANTHER automatically determines the run validity based on criteria in Table 2.

Table 2: Acceptance Criteria of Positive and Negative Calibrators

Calibrator	RLU	<i>M. genitalium</i> Result
Negative Calibrator Analyte	≥ 0 and $\leq 40,000$	Valid
Negative Calibrator IC	$\geq 120,000$ and $\leq 425,000$	Valid
Positive Calibrator Analyte	$\geq 650,000$ and $\leq 2,700,000$	Valid
Positive Calibrator IC	≥ 0 and $\leq 800,000$	Valid

A run may be invalidated by an operator if technical, operator, or instrumental difficulties are observed and documented while performing the assay. An invalid run must be repeated. Aborted runs must be repeated.

B. Reducing RNA Contamination

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. RNA contamination can be minimized by performing daily, weekly, and monthly maintenance.

1) Maintenance: Daily

- a. Laboratory bench surfaces must be decontaminated regularly with household bleach diluted 1:1 (1-part bleach, 1-part water). Allow bleach to contact surfaces for at least 1 minute, then follow with water to rinse. Do not allow the bleach to dry. Chlorine solutions may damage metal.
- b. Submerge Reagent and Specimen Racks in household bleach diluted 1:1, ensuring they are covered by the bleach solution. Keep the racks submerged for 10 minutes. Longer exposure will damage the racks. Rinse the racks thoroughly with water, then dry the racks completely with paper towels.

2) Maintenance: Weekly

- a. The 2 weekly maintenance items are to change the sample shield and a PC reboot.
- b. Under the “Tasks” screen, select “perform maintenance”.
- c. Select the needed maintenance item and select start.
- d. Follow instructions on screen. Remember to change gloves after each step. If any problems occur or observations are noted, make a note in the comment section of the software before clicking “done”.
- e. For the sample shield: using gloves push the sample shield towards the back of the machine and lift it off the silver pins. Place in a bin of 2.5-3.5% sodium hypochlorite for at least 10 minutes. Change gloves and place a clean, dry sample shield in place making sure both pins are visible and pull forward. Once the used sample shield has been in the bleach solution for at least 10 minutes, rinse it with tap water and allow to dry before storing.
- f. For PC reboot; remove all assay reagents and samples from the machine and start the process following the instructions on the screen. The machine will reboot automatically. After it restarts you must log in again and it must be primed before use.

3) Maintenance: Monthly

- a. Once a month the entire machine is cleaned with 2.5-3.5% sodium hypochlorite letting it remain for 1 minute then follow with a distilled water rinse. The tips are replaced, and the waste is emptied, and the drawer cleaned. The bulk fluid bottles in the universal fluid drawer are wiped and the connectors are cleaned and rinsed. The complete instructions for monthly cleaning are accessed

through the maintenance selection on the “Tasks” screen of the computer. This procedure takes about 240 minutes.

11) REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET

ACCEPTABLE CRITERIA

Repeat run for individual sample.

12) LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

1. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this procedure may result in erroneous results.
2. The effects of tampon use, douching, and specimen collection variables have not been evaluated for their impact on the detection of *M. genitalium*. Reliable results are dependent on adequate specimen collection, transport, storage, and processing. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary.
3. Results from the APTIMA *Mycoplasma genitalium* assay should be interpreted in conjunction with other clinical data available to the clinician.
4. 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.
5. The APTIMA *M. genitalium* 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.
6. Performance using any female specimen types has not been determined in pregnant women.
7. Performance of the assay has not been evaluated in women less than 19 years of age.
8. If a specimen has a small number of *M. genitalium* organisms, uneven distribution of these organisms may occur, which may affect the ability to detect *M. genitalium* rRNA in the collected material. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary.

13)REFERENCE RANGES (NORMAL VALUES)

All normal non-infected humans should have negative values.

14)CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15)SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25 °C during preparation and testing for up to 4 hours.

16)ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

The samples remain frozen until the system is operating.

17)TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

18)TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

- A.** Test results are documented through the lab management database. Generally, a CDC epidemiologist communicates the findings to other participants in the study. Final reports may be electronic or in printed form.
- B.** All electronically held data are backed up routinely.
- C.** Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data.

19)SUMMARY STATISTICS AND QC GRAPHS

Qualitative assays, such as the APTIMA *M. genitalium* assay, provide positive, negative or borderline/indeterminate results. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

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