Laboratory Procedure Manual

Analyte:	*Chlamydia trachomatis*
Matrix:	Urine
Method:	Hologic APTIMA Combo 2 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.

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

Data File Name	Variable Name	SAS Label
CHLA_L_R CHLM_L_R	URXUCL	Chlamydia, urine

1) SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Chlamydia trachomatis (CT) is a common sexually transmitted bacterium, with 1.6 million new cases in the United States reported to the Centers for Disease Control and Prevention in 2016. The APTIMA Combo 2 Assay is a nucleic acid amplification test (NAAT) that utilize target capture for the *in vitro* qualitative detection and differentiation of ribosomal RNA (rRNA) from CT and/or *Neisseria gonorrhoeae* (GC). The APTIMA Combo 2 Assay combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) to streamline specimen processing, amplify target rRNA, and detect amplicon, respectively.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the rRNA targets and protect them from degradation during storage. When the APTIMA Combo 2 Assay is performed in the laboratory, the target rRNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification. Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The APTIMA Combo 2 Assay replicates a specific region of the 23S rRNA from CT and a specific region of the 16S rRNA from GC via DNA intermediates. A unique set of primers is used for each target molecule. Detection of the rRNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent DNA probes, which are complementary to

a region of each target amplicon, are labeled with different acridinium ester molecules. The labeled DNA probes combine with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the CT and GC labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for CT signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for GC signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

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 Combo 2 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 Combo 2 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 2ºC to 30ºC for up to 24 hours before urine is transferred to the transport tube. Processed urine in the transport tube is stored at 2ºC to 30ºC 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 Combo 2 Assay Kit
2. APTIMA Combo 2 Control 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 (2ºC to 8ºC) and corresponding reconstitution solutions (15ºC to 30ºC) 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 45º 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 (15ºC to 30ºC) and TCR-B Reagent (2ºC to 8ºC) from storage.

b. Check the lot number on the TCR bottle and TCR-B 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 TCR-B Reagent 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 Reagent bottle and cap.

3) Selection Reagent preparation:

a. Remove the Selection Reagent from storage (2ºC to 30ºC). 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 (2ºC to 8ºC). Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15ºC to 30ºC) prior to the start of the assay.

b. If reconstituted Probe Reagent contains precipitate at room temperature (15ºC to 30ºC), heat the capped bottle at a temperature that does not exceed 62ºC 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

Not applicable.

E. CONTROLS

1) The APTIMA Negative Control for CT, and the APTIMA Positive Control for CT, act as controls for the target capture, amplification, and detection steps of the assay

a. The APTIMA Positive Control for CT contains non-infectious *C. trachomatis* rRNA

b. To work properly with the PANTHER system software, one pair of controls is required

c. Each APTIMA control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

7) CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Not applicable.

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 controls.

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. Controls 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

a. Not applicable

C. Interpretation of Results

Assay test results are automatically interpreted by the PANTHER system APTIMA Combo 2 Assay software using the APTIMA Combo 2 protocol and presented as individual CT and GC test results. A test result may be negative, positive, equivocal, or invalid as determined by the kinetic type and total Relative Light Unit (RLU) in the detection step (Table 1). A test result may be invalid due to a parameter outside the normal expected ranges. Initial equivocal and invalid test results should be retested.

Table 1: Test Results Based on RLU Range

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

9) REPORTABLE RANGE OF RESULTS

Positive, negative, equivocal, or invalid are the initial range of results based on assay results with interpretations in Table 3. Initial equivocal and invalid test results should be repeated and interpreted based on parameters in Table 4. Only positive, negative, or invalid results are reported.

Table 3: Initial Results and Interpretations

CT Test Result	Interpretation
CT Positive	Positive for CT rRNA
CT Negative	Presumed negative for CT rRNA
CT Equivocal	Sample should be retested
Invalid	Sample should be retested

Table 4: Retest Results and Interpretations

CT Test Result	Interpretation
CT Positive	Positive for CT rRNA
CT Negative	Presumed negative for CT rRNA
CT Equivocal	Indeterminate, a new specimen should be collected
Invalid	Indeterminate, a new specimen should be collected

A. Note: 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

B. Note: The first valid result for each analyte is the result that should be reported.

10) QUALITY CONTROL (QC) PROCEDURE

A. Positive & Negative Controls

The Positive Control (CT+/GC-) and the Negative Control, (GC+/CT-) act as controls for the target capture, amplification, and detection steps of the assay. The Positive Control serves as the negative control for the GC test results. The Negative Control serves as the positive control for the GC test results. Software on the PANTHER automatically validates specimen results based on control criteria in Table 2.

Table 2: Results of Positive and Negative Controls for a Valid Test

Control	Total RLU (x1000)	*C. trachomatis* Result
Positive Control	≥100 and < 3,000	Positive
Negative Control	≥ 150 and < 3,000	Negative

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 changer 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. Careful consideration of performance data is recommended for interpreting APTIMA Combo 2 Assay results for asymptomatic individuals in low prevalence populations.
2. Performance for detecting CT 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.
3. A negative result does not preclude the presence of a CT infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient rRNA to be detected. Test results may be affected by improper specimen collection, improper specimen storage, technical error, or specimen mix-up.
4. As is true for all non-culture methods, a positive specimen obtained from a patient after therapeutic treatment cannot be interpreted as indicating the presence of viable CT.

5. As is true for all urine test methods, a negative urine result for a female patient who is clinically suspected of having a chlamydial infection does not rule out the presence of CT in the urogenital tract. Testing of an endocervical specimen is recommended in such cases.
6. 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.
7. The effects of tampon use, douching, and specimen collection variables have not been evaluated for their impact on the detection of *C. trachomatis*.
8. 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.
9. Therapeutic failure or success cannot be determined with the APTIMA Combo 2 Assay since nucleic acid may persist following appropriate antimicrobial therapy.
10. Results from the APTIMA Combo 2 Assay should be interpreted in conjunction with other clinical data available to the clinician.
11. The APTIMA Combo 2 Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the number of organisms in a specimen.
12. The performance of the PANTHER system has not been determined at altitudes above 2000 meters (6561 feet).
13. Performance of the assay has not been evaluated in women less than 16 years of age.

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

B. 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.

C. All electronically held data are backed up routinely.

D. 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 Combo 2 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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