

Laboratory Procedure Manual

Analyte: Chlamydia trachomatis

Matrix: **Urine**

Method: C. trachomatis Assay Using Gen-Probe
APTIMA Combo

Method No.:

First Published:

Revised:

as performed by: *Division of AIDS, STD, and TB Laboratory Research
National Centers for Infectious Diseases
National Centers for Disease Control and Prevention*

Contact:

Important Information for Users

The Division of AIDS, STD, and TB Laboratory Research 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

CHLMDA_I

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

Variable Name	SAS Label
URXUCL	Chlamydia, urine

1. SUMMARY OF TEST PRINCIPLE

The GEN-PROBE APTIMA Combo 2 Assay combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

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 the use of capture oligomers in a method called target capture; magnetic microparticles are another key feature of target capture. 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 GEN-PROBE APTIMA Combo 2 Assay reaction 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.

SAFETY PRECAUTIONS

For *in vitro* diagnostic use.

The assay was not evaluated in patient populations with a low prevalence of CT disease; therefore, performance in low prevalence settings has not been determined.

Use only supplied or specified disposable laboratory ware.

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.

Warning: Irritants and Corrosives: Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash the 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 1:1 dilution of bleach (1 part bleach, 1 part water).

To help prevent lab areas from becoming contaminated with amplicon, 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.

This method has been tested using endocervical and male urethral swab specimens, vaginal swab specimens, PreservCyt liquid Pap specimens, rectal and oropharyngeal swabs, female and male urine specimens only. Performance with other specimens has not been assessed. Specimens other than those collected with the following specimen collection kits have not been evaluated:

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens (also used for rectal and oropharyngeal specimens)
- APTIMA Urine Collection Kit for Male and Female Urine Specimens
- APTIMA Vaginal Swab Specimen Collection Kit

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. Otherwise, the specimen must be rejected. 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.

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.

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 come in contact with specimen.

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

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

Do not use this kit after its expiration date. **Do not** interchange, mix, or combine reagents from kits with different lot numbers.

2. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- a. Specimens received from various research studies labeled by the specimen ID, collection date, and type of sample (i.e. urine). 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 and the final values are approved by the reviewing supervisor for release, all results are entered onto the specific study data file.

3. Procedures for Procedures for Collecting, Storing, and Handling Specimens; Criteria for Specimen Rejection

- a. For *in vitro* diagnostic use.
- b. The assay was not evaluated in patient populations with a low prevalence of CT disease; therefore, performance in low prevalence settings has not been determined.
- c. Use only supplied or specified disposable laboratory ware.
- d. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- e. **Warning: Irritants and Corrosives:** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash the affected area with water. If these fluids spill, dilute the spill with water before wiping it dry.
- f. Work surfaces, pipettes, and other equipment must be regularly decontaminated with a 1:1 dilution of bleach (1 part bleach, 1 part water).
- g. A separate area for DKA is strongly recommended to minimize amplicon contamination in the assay. This dedicated area should be away from the reagent preparation, target capture, and amplification area.
- h. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a unidirectional workflow: from reagent preparation through DKA. 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.
- i. This method has been tested using endocervical and male urethral swab specimens, vaginal swab specimens, PreservCyt liquid Pap specimens, rectal and oropharyngeal swabs, female and male urine specimens only. Performance with other specimens has

not been assessed. Specimens other than those collected with the following specimen collection kits have not been evaluated:

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens (also used for rectal and oropharyngeal specimens)
 - APTIMA Urine Collection Kit for Male and Female Urine Specimens
 - APTIMA Vaginal Swab Specimen Collection Kit
- j. 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. Otherwise, the specimen must be rejected.
- k. 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.
- l. 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.
- m. 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 come in contact with specimen.
- n. If the lab receives a **swab** specimen transport tube containing no swab, two swabs, or a swab not supplied by Gen-Probe, the specimen must be rejected. Prior to rejecting a swab transport tube with no swab, verify that it is not an APTIMA □ Specimen Transfer Tube as this specimen transport will not contain a swab.
- o. Upon piercing, liquid can discharge from APTIMA transport tube caps under certain conditions. Follow instructions in *Target Capture, Rack Setup*, step 3, to prevent this occurrence.
- p. The performance of vaginal swab specimen has not been evaluated in pregnant women.
- q. The performance of vaginal swab and PreservCyt liquid Pap specimens has not been evaluated in women less than 16 years of age.
- r. Do not use this kit after its expiration date. **Do not** interchange, mix, or combine reagents from kits with different lot numbers.
- s. Tips with hydrophobic plugs must be used. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the **Target Capture** and **Amplification** steps, and one for use in the DKA steps. Two micropipettors must be dedicated for use in this assay: one for use in specimen transfer and one for use in reagent preparation. All pipettors must be cleaned regularly.
- t. When using repeat pipettors for reagent addition, do not touch the tube with the pipette tip to prevent carryover from one tube to another.
- u. Adequate mixing is necessary to achieve accurate assay results.

- v. Separate water baths must be dedicated for the target capture, amplification, and DKA steps in the assay.

4. Procedures for Microscopic Examinations; Criteria for Rejecting Inadequately Prepared Slides

Not applicable for this procedure.

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

Instrumentation

- (1) Panther system
- (2) Multi-tube vortex mixer
- (3) Circulating water bath

Other Materials

- (1) Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL)
- (2) Micropipettor: 20 μ L to 200 μ L
- (3) Tips, Pipetman P1000 Style, APTIMA Combo 2
- (4) Tips, 1000 μ L
- (5) Pipette tips 20 μ L to 200 μ L
- (6) Household bleach (sodium hypochlorite solution)
- (7) APTIMA® Auto Detect Kit
- (8) APTIMA® Controls Kit
- (9) APTIMA® Penetrable Caps
- (10) Gloves

Reagent Preparation

- (1) To reconstitute the APTIMA Combo 2 Enzyme, Amplification, and Probe Reagents:
 - a) Pair the appropriate reconstitution solution with the dried reagent. The labels have been color coded so that they can be paired correctly.
 - b) Open the dried reagent and firmly insert the notched end of the reconstitution collar into the glass vial.
 - c) Open the reconstitution solution (save the cap) and, while holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle.
 - d) Invert the assembly, allow the solution to drain into the glass container, then gently swirl the solution within the container. Invert the assembly and tilt it at a 45° angle. Allow all of the liquid to drain back into the plastic bottle.

- e) Remove the reconstitution collar and the glass vial.
 - f) Discard both the reconstitution collar and glass vial.
 - g) Recap the plastic bottle and peel away the top label on the reconstituted reagent. Record required information on the remaining bottle label.
 - h) Discard reconstituted reagent after 30 days or by the expiration date, whichever comes first.
- (2) Previously reconstituted Probe, Amplification, and Enzyme Reagents, must reach room temperature (15°C to 30°C) prior to the start of an assay. If the Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C for 1 to 2 minutes. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading it onto the system.
- (3) **Note:** This inversion step should be performed any time that the precipitate is being brought into solution, whether by heating at 62°C or by warming at room temperature.
- (4) Prepare the Target Capture Reagent plus Target Capture Reagent B (TCR plus TCR-B) as follows:
- a) Determine the number of reactions to be performed (specimens plus controls).
 - b) Calculate the volumes of Target Capture Reagent (TCR) and Target Capture Reagent B (TCR-B) as follows
Volume of TCR (mL) = (number of reactions + 5 extra reactions) x 0.1 mL
Volume of TCR-B (mL) = Volume of TCR (mL)/100
 - c) Transfer the calculated volume of TCR to an appropriately sized, dedicated, clean, dry container and, using a micropipettor, add the calculated volume of TCR-B into the TCR.
 - d) Thoroughly mix the solution by swirling.
 - e) The TCR plus TCR-B is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

Standards Preparation

a. Calibration Standard

Not applicable for this procedure. Sample results are automatically compared against predetermined cut-off values set by the manufacturer.

Preparation of Quality Control Materials

a. negative Control

Prepackaged and ready to use.

b. Positive Control

Prepackaged and ready to use.

Reagents

Each APTIMA COMBO 2 Reagent Pack contains a refrigerated and non-refrigerated box:

Refrigerated Box (2°C to 8°C):

APTIMA Combo 2 Enzyme Reagent
Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.

APTIMA Combo 2 Amplification Reagent
Nucleic acids dried in buffered solution containing < 5% bulking agent.

APTIMA Combo 2 Probe Reagent
Non-infectious chemiluminescent DNA probes (< 500 ng/vial) dried in succinate buffered solution containing < 5% detergent.

APTIMA Combo 2 Target Capture Reagent B
Non-infectious nucleic acid in a buffered solution containing < 5% detergent.

APTIMA Positive Control, CT/Negative Control, GC
Non-infectious C. trachomatis nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 1 C. trachomatis IFU (5 fg/assay).*

APTIMA Positive Control, GC/Negative Control, CT
Non-infectious N. gonorrhoeae nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 50 N. gonorrhoeae cells (250 fg assay).*

APTIMA Combo 2 Amplification Reconstitution Solution
Aqueous solution containing preservatives.

APTIMA Combo 2 Enzyme Reconstitution Solution
HEPES buffered solution containing a surfactant and glycerol.

APTIMA Combo 2 Probe Reconstitution Solution
Succinate buffered solution containing < 5% detergent.

Non-Refrigerated Box (15°C to 30°C):

APTIMA Combo 2 Target Capture Reagent
Buffered salt solution containing solid phase (< 0.5 mg/ml) and capture oligomers.

APTIMA Wash Solution mL
10 mM HEPES buffered solution containing < 2% detergent.

APTIMA Buffer for Deactivation Fluid
800 mM bicarbonate buffered solution.

APTIMA Oil Reagent
Silicone oil.

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

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

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

Controls are stable until the date indicated on the vials.

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

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

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

Do not freeze reagents .

6. Calibration and Calibration-Verification Procedures

a. Calibration Curve

Not applicable

b. Calibration Verification

Not applicable

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Equipment Preparation

1. Adjust one water bath to $62^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (for target capture, and primer annealing), a second water bath to $42^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (for amplification), and a third water bath to $62^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (for DKA).
2. Prior to starting the assay, wipe down work surfaces and pipettors with household bleach diluted 1:1 with water (one part bleach, one part water). Allow the bleach to contact surfaces and pipettors for at least one minute and then follow with a water rinse. Do not allow the bleach to dry. Cover the bench surface on which the test will be performed with clean, plastic backed absorbent laboratory bench covers.
3. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with APTIMA Wash Solution and the aspirator is connected to the vacuum pump.

B. Reagent Preparation

To reconstitute the APTIMA Combo 2 Enzyme, Amplification, and Probe Reagents:

Pair the appropriate reconstitution solution with the dried reagent. The labels have been color coded so that they can be paired correctly.

Open the reconstitution solution (save the cap) and, while holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle.

Invert the assembly, allow the solution to drain into the glass container, then gently swirl the solution within the container. Invert the assembly and tilt it at a 45° angle.

Allow all of the liquid to drain back into the plastic bottle.

Remove the reconstitution collar and the glass vial.

Discard both the reconstitution collar and glass vial.

Recap the plastic bottle and peel away the top label on the reconstituted reagent.

Record required information on the remaining bottle label.

Discard reconstituted reagent after 30 days or if they have been on board the PANTHER for 72 hours or longer, or by the expiration date, whichever comes first.

Previously reconstituted Probe, Amplification, and Enzyme Reagents, must reach room temperature (15°C to 30°C) prior to the start of an assay. If the Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C for 1 to 2 minutes. Mix Probe Reagent by gentle inversion, being careful not to induce foam, prior to loading it onto the system.

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

Prepare the Target Capture Reagent plus Target Capture Reagent B (TCR plus TCR-B). Assure that there are no bubbles in reagents. Load on board the PANTHER at appropriate time .

C. Standards Preparation

Calibration Standard Not applicable for this procedure. Sample results are automatically compared against predetermined cut-off values set by the manufacturer.

D. Preparation of Quality Control Materials

- Negative Control: Prepackaged and ready to use.
- Positive Control: Prepackaged and ready to use .

E. Rack Setup

Do not vortex specimens.

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

Inspect transport tubes:

- If a transport tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF (Relative Centrifugal Force) to eliminate the bubbles
- If a transport 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.
- If the liquid level is not between the two black indicator lines on the urine transport tube label, the specimen must be rejected. Do not pierce an overfilled tube.
- If a urine specimen contains precipitates, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, ensure that the precipitate does not prevent delivery of the specimen.

Note: Failure to follow steps 3a-c may result in liquid discharge from the transport tube cap.

Load specimens and controls into PANTHER specimen racks.

Place sample rack retainer over each rack and load onto PANTHER at appropriate time.

To work properly with the APTIMA Assay software, the Positive Control and Negative Control barcodes must be visible through the rack

F. Operating the PANTHER System:

Before actually loading reagents and samples onto the PANTHER, perform instrument and laboratory check by recording room temperature (15-30C) to make sure it falls within the acceptable ranges if it has not already been done. Perform an external inspection of machine and check for any leaks.

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.

Prepare reagents as instructed if they are not already prepared. Remove gloves. Log on to PANTHER software using your login and password.

Exit power save mode.

Wearing clean gloves, load tips and MTU's if needed.

Load universal fluids if necessary.

Empty waste from waste drawer if needed. Change gloves.

Ensure all maintenance is current; system will not operate if any maintenance is overdue.

Prime if needed.

Load assay reagents that are prepared as instructed above, making sure there are no bubbles in the reagents.

Load samples, including controls. Controls are valid for 24 hours as long as reagents are not removed from the system. Any time reagents are replaced or 24 hours have passed, control must be added.

Change gloves.

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 operators manual or on-screen instructions.

Return to system to load tips, MTU's, additional reagents and additional samples as needed. Change gloves between each task.

Samples may be removed when pipetting is complete and all amle are indicated in blue on the screen graphic. Controls are disposed of in a biohazard bag or pan to prevent contamination. Make sure to keep tubes upright at all times.

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.

When run is complete, print the report "results by work list".

Reagent racks should be rinsed in the bin of 2.5-3.5% sodium hypochlorite solution and rinsed as were sample racks. Change gloves.

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 temp.

G. Method Performance Specifications

Controls

To work properly with the PANTHER APTIMA Assay software, one pair of controls is required. The Positive CT Control/Negative GC control is one tube and the Positive GC Conyrol/CT Negative control is the second control tube. These 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 two following conditions has been met:

A pair of controls is currently being processed by the system.

Valid results for the controls are registered on the system.

Once the control tubes have been pipetted and are processed for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:

Controls results are invalid.

The associated reagent kit is removed from the system.

The associated assay reagent kit has exceeded stability limits.

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

Temperature

Room temperature is defined as 15-30 C.

Glove powder

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

Powderless gloves are recommended.

H. Monitoring for the Presence of DNA Contamination

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 the Endocervical and Male Urethral Swab Specimens:

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

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

Immediately insert the swab into transport tube.

Carefully break the swab shaft at the score line; avoid splashing of the contents.

Recap the swab transport tube tightly.

Repeat Steps 2 to 5 for all areas to be swabbed.

Test the swab using the APTIMA Combo 2 Assay as described in Section 6.

Record environmental contamination.

I. Target Capture

1. Cover the TTUs with sealing cards and shake the rack gently by hand. Do not vortex. Incubate the rack at $62^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in a water bath for 30 ± 5 minutes.
2. Remove the rack from the water bath and blot the bottoms of the tubes dry on absorbent material.
3. Ensure the sealing cards are firmly seated. If necessary, replace them with new sealing cards and seal the TTUs tightly.
4. Vortex the rack for 60 seconds on the multi-tube vortex mixer. Begin vortexing within 2 minutes of removal of the rack from the water bath.
5. Without removing the sealing cards, incubate the rack at room temperature for 30 ± 5

minutes.

6. Place the rack on the TCS magnetic base for 5 to 10 minutes.
7. Prime the dispense station pump lines by pumping APTIMA Wash Solution through the dispense manifold. Pump enough liquid through the system so that there are no air bubbles in the line and all ten nozzles are delivering a steady stream of liquid.
8. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the aspiration manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification. It may take 15 seconds to achieve this reading. Reconnect the manifold, and ensure that the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed.
9. Firmly attach the aspiration manifold to the first set of tips. Aspirate all liquid by lowering the tips into the first TTU until the tips come into brief contact with the bottoms of the tubes. Do not hold the tips in contact with the bottoms of the tubes.
10. After the aspiration is complete, eject the tips into their original tip cassette. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each specimen.
11. Place the dispense manifold over each TTU and, using the dispense station pump, deliver 1.0 mL of APTIMA Wash Solution into each tube of the TTU.
12. Cover the tubes with a sealing card and remove the rack from the TCS. Vortex the rack once on the multi-tube vortex mixer.
13. Place the rack on the TCS magnetic base for 5 to 10 minutes.
14. Aspirate all liquid as in Steps 8 and 10.
15. After the final aspiration, remove the rack from the TCS base and visually inspect the tubes to ensure that all liquid has been aspirated, and all tubes contain magnetic particle pellets. If any liquid is visible, place the rack back on the TCS base for 2 minutes, and repeat the aspiration for that TTU using the same tips used previously for each specimen. If ANY magnetic particle pellet is visible after aspiration is completed, the tube may be accepted. If no pellet is visible, the specimen should be retested. If the same specimen does not contain a magnetic particle pellet at this step in a subsequent run, this may indicate a specimen-specific problem. Re-collection of the specimen is recommended in this situation.

J. Amplification

1. Using the repeat pipettor, add 75 μ L of the reconstituted Amplification Reagent to each reaction tube. All reaction mixtures in the rack should now be red.
2. Using the repeat pipettor, add 200 μ L of Oil Reagent.
3. Cover the tubes with a sealing card and vortex them on the multi-tube vortex mixer.
4. Incubate the rack in a water bath at $62^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 10 ± 5 minutes.
5. Transfer the rack into a water bath at $42^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 ± 2 minutes.
6. With the rack in the water bath, carefully remove the sealing card and, using the repeat pipettor, add 25 μ L of the reconstituted Enzyme Reagent to each of the reaction mixtures. All reactions should now be orange.

7. Immediately cover the tubes with a fresh sealing card, remove the rack from the water bath, and mix the reactions by gently shaking the rack by hand.
8. Incubate the rack at $42^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 60 ± 15 minutes.

K. Dual Kinetic Assay

1. Hybridization

- a. Remove the rack from the water bath and transfer to the DKA area. Add 100 μL of the reconstituted Probe Reagent, using the repeat pipettor. All reaction mixtures should now be yellow.
- b. Cover the tubes with a sealing card and vortex the rack on the multi-tube vortex mixer.
- c. Incubate the rack in a $62^{\circ}\text{C} \pm 1^{\circ}\text{C}$ water bath for 20 ± 5 minutes.
- d. Remove the rack from the water bath and incubate at room temperature for 5 ± 1 minute.

2. Selection

- a. Using the repeat pipettor, add 250 μL of Selection Reagent to each tube. All reactions should now be red.
- b. Cover the tubes with a sealing card, vortex the rack for 10 seconds or until the color is uniform, and incubate the rack in a water bath at $62^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 10 ± 1 minutes.
- c. Remove the rack from the water bath.

3. Detection

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

- a. Incubate the rack at 18°C to 28°C for 15 ± 3 minutes.
Note: This temperature range is critical for assay performance.
- b. Prepare the LEADER HC+ Luminometer by placing one empty TTU in cassette position number 1 and performing the WASH protocol.
- c. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the tests.
- d. Load the TTUs into the luminometer.
- e. Log on to the computer. Click on **NEW RUN** and enter the number of tubes (controls and specimens). Click **NEXT** to begin the run.
Note: The run must be completed within 2 hours of the end of the selection step incubation.
- f. Prepare a buffered bleach deactivation solution by mixing equal volumes of household bleach and APTIMA Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. This buffered bleach solution is stable for four weeks at room temperature.

- g. After removing the used TTUs from the luminometer, place the TTUs into the container with the buffered bleach solution. Allow the TTUs to sit in the container for 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

L. Maintenance: Weekly

The 2 weekly maintenance items are to change the sample shield and a pc reboot. Under the “Tasks” screen, select “perform maintenance”.

Select the needed maintenance item and select start.

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”.

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 drying before storing.

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

M. Maintenance: Monthly

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.

For troubleshooting see the PANTHER system manual on the computer or call technical support 1-888-484-4747. PANTHER serial number: 00736

L. Interpretation of Test Results

Assay test results are automatically interpreted by the APTIMA Assay software, using the APTIMA Combo 2 protocol, and presented as individual CT test results. A test result may be negative, equivocal, positive, or invalid as determined by the kinetic type and total RLU in the detection step (see the following tables). A test result may be invalid due to a parameter outside the normal expected ranges. Initial equivocal and invalid test results should be repeated.

Kinetic Type	Total RLU (x1000) to give CT Result		
	Negative	Equivocal	Positive
CT only	1 to <25	25 to <100	100 to <4,5000

CT indeterminate	1 to <85	85 to <4,500	NA
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Kinetic Type	Total RLU (x1000) to give CT Result		
	Negative	Equivocal	Positive
GC only	1 to <60	60 to <150	150 to <4,5000
CT and GC	1 to <85	85 to <250	250 to < 4,500

9. REPORTABLE RANGE OF RESULTS

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

10. QUALITY CONTROL (QC) PROCEDURES

Controls must be run with each assay. The APTIMA Positive Control CT/Negative Control GC and the APTIMA Positive Control GC/Negative Control CT act as controls for the **Target Capture, Amplification, and Detection** steps of the assay. The Positive Control, CT/Negative Control, GC serves as the negative control for the GC test results. The Positive Control, GC/Negative Control, CT serves as the negative control for the CT test results. Correct preparation of specimens is confirmed visually by the presence of a single GEN-PROBE collection swab in a swab specimen transport tube, or a final volume of urine in between the black fill lines of a urine specimen transport tube.

Control	Total RLU (x1000)	CT Result
Positive Control CT / Negative Control GC	≥100 and <3,000	CT Positive
Positive Control GC / Negative Control CT	≥150 and <3,000	CT Negative

Assay test results are automatically interpreted by the PANTHER System APTIMA Trichomonas Assay software. A test results may be negative, positive or invalid as determined by total RLU in the detection step. 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.

Specimen Processing Controls

Specimen processing controls may be tested in accordance with the requirements of appropriate accrediting organizations. A positive control should test the entire assay system. For this purpose, known positive specimens can serve as controls by being processed and tested in conjunction with unknown specimens. Specimens used as

processing controls must be stored, processed, and tested according to the package insert. Specimen processing controls which simulate urine processing can also be prepared as described below.

Chlamydia trachomatis:

If a known positive specimen is not available, another approach is to assay a stock culture of *C. trachomatis* LGV2 (ATCC VR-902B) prepared as described below:

1. Thaw a vial of *C. trachomatis* LGV2 cells ATCC VR-902B.
2. Prepare 10-fold serial dilutions to a 10^5 dilution (at least 5 mL final volume) in phosphate buffered saline (PBS).
3. Place 4 mL of 10^5 dilution in an APTIMA Unisex Swab Specimen Collection Kit for the Endocervical and Male Urethral Swab tube.
4. Process the sample as described in Section 6.

Monitoring for the Presence of DNA Contamination

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 the Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the swab transport media 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; avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for all areas to be swabbed.
7. Test the swab using the APTIMA Combo 2 Assay as described in Section 6.

Record environmental contamination.

Decontamination

1. Surfaces and Pipettors (performed daily)

Laboratory bench surfaces and pipettors must be decontaminated regularly with household bleach diluted 1:1 with water, (1 part bleach, 1 part water). Allow the bleach to contact surfaces for at least 1 minute, and then follow with a water

rinse. **Do not allow the bleach to dry.** Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment with water to avoid pitting.

2. TCS Manifold (performed monthly)

Disconnect the aspiration manifold by removing the tube from the tube attachment. Submerge the manifold in household bleach diluted 1:1 with water, ensuring that the handles and manifold submerged for 10 minutes. Longer exposure will damage the manifold. Rinse the manifold thoroughly with water, and then dry it completely with paper towels. Ensure that the area under the ejector plate is dry.

3. TCS Waste Container (performed monthly)

Disconnect the waste bottle from the unit and pour the waste into a sink. Add 400 mL of bleach to the bottle. Leaving the bleach in the bottle, reconnect the bottle to the unit. Reconnect the manifold and run the pump for 3 minutes to complete the drying process.

4. TCS Unit (performed monthly)

Wipe the surfaces of the TCS unit and Wash Buffer ejector tips with paper towels moistened with bleach diluted 1:1 with water. Follow the bleach step with a water rinse, and then dry the surfaces completely with paper towels.

5. Racks (performed daily)

Submerge the racks in household bleach diluted 1:1 with water, ensuring that 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, and then dry the racks completely with paper towels.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Repeat run for individual sample.

12. LIMITATIONS OF THE PROCEDURE

1. Swab specimens were evaluated in the APTIMA Combo 2 Assay for interference by blood, gynecological lubricants, and spermicidal. Urine specimens were evaluated for interference by blood, commonly used vitamins, minerals, and over-the-counter pain relievers. The data indicated no assay interference by these substances.
2. The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of CT or GC.
3. The presence of mucus in samples does not interfere with the detection of CT or GC by the APTIMA Combo 2 Assay. However, to ensure collection of cells infected with CT, columnar epithelial cells lining the endocervix should be sampled. If excess mucus is not removed, sampling of these cells is not ensured.
4. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this insert may result in erroneous results.
5. This method has been tested using only the following specimens:
Clinician-collected endocervical, vaginal and male urethral swab specimens

- Clinician-collected PreservCyt liquid Pap specimens
 - Patient-collected vaginal swab specimens
 - Patient-collected female and male urine specimens
6. Performance with other specimens has not been assessed. Specimens other than those collected with the following specimen collection kits have not been evaluated:
 - APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens
 - APTIMA Urine Collection Kit for Male and Female Urine Specimens
 - APTIMA Vaginal Swab Specimen Collection Kit
 - PACE Specimen Collection Kit for Urethral or Conjunctival Specimens (in conjunction with the APTIMA Adapter Kit)
 - PACE Specimen Collection Kit for Endocervical Specimens (in conjunction with the APTIMA Adapter Kit)
 - APTIMA Specimen Transfer Kit (for use with gynecologic samples processed with the Cytoc ThinPrep 2000 System)
 7. Vaginal swab, PreservCyt liquid Pap, and urine sampling are not designed to replace cervical exams and endocervical samples for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.
 8. The APTIMA Combo 2 Assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications. For those patients for whom a false positive result may have adverse psycho-social impact, the CDC recommends retesting (4).
 9. Reliable results are dependent on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary.
 10. Therapeutic failure or success cannot be determined with the APTIMA Combo 2 Assay since nucleic acid may persist following appropriate antimicrobial therapy.
 11. Results from the APTIMA Combo 2 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
 12. 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, or specimen mix-up.
 13. 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.
 14. For the vaginal swab, endocervical swab, male urethral swab and urine specimen clinical studies, performance characteristics for detecting CT and GC are derived from high prevalence populations. Positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.

15. For the PreservCyt liquid Pap specimen clinical study, the APTIMA Combo 2 Assay performance for detecting CT and GC is derived primarily from low prevalence populations. Nonetheless, positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.
16. Patient collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated.
17. The patient-collected vaginal swab specimen application is limited to health care facilities where support/ counseling are available to explain the procedures and precautions.
18. The APTIMA Combo 2 Assay has not been validated for use with vaginal swab specimens collected by patients at home.
19. The performance of the vaginal swab specimen has not been evaluated in pregnant women.
20. The performance of vaginal swab, PreservCyt liquid Pap specimen has not been evaluated in women less than 16 years of age.

13. REFERENCE RANGES (NORMAL VALUES

All normal noninfected 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. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

The samples are 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

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.

All electronically held data are backed up routinely.

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 are qualitative assays with a positive, negative or borderline/indeterminate result. 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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