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

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

Data File Name	Variable Name	SAS Label
CHLMDA_G	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.

CLINICAL SIGNIFICANCE

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are the most common sexually transmitted bacterial diseases in the United States. Approximately 4 million new chlamydia cases are estimated to occur each year in the United States with worldwide estimates of approximately 50 million new cases annually (1-3). The incidence of

chlamydial infections in women in the US in 1996 was 186.6 per 100,000. The total number of chlamydial infections and gonorrhea cases reported in the US in 1996 were 490,080 and 325,883, respectively (2).

Chlamydiae are gram-negative, obligate intracellular bacteria. They form characteristic intracellular inclusions which can be observed in cell culture by light microscopy after special staining is applied (4). *Chlamydia trachomatis* causes cervicitis, urethritis, salpingitis, proctitis and endometritis in women and urethritis, epididymitis and proctitis in men. Acute infections are reported more frequently in men because women often have no symptoms of infection. It has been estimated that 70 - 80% of women and up to 50% of men who are infected experience no symptoms. Many chlamydial infections in women remain untreated which may result in low-grade inflammation in the Fallopian tubes, a leading contributor to infertility. This organism can also be transmitted in the birth canal, potentially resulting in infant conjunctivitis and/or chlamydial pneumonia in newborns (4, 5).

Neisseria gonorrhoeae are gram-negative, oxidase positive diplococci which can be observed in Gram-stained smears of urethral discharges, usually within neutrophils. Culture of *N. gonorrhoeae* can be difficult because the organism does not survive long outside its host and is highly susceptible to adverse environmental conditions such as drying and *extreme* temperatures (6). *Neisseria gonorrhoeae* causes acute urethritis in males, which if untreated can develop into epididymitis, prostatitis, and urethral stricture. In females, the primary site of infection is the endocervix. An important complication in females is development of pelvic inflammatory disease which contributes to infertility (7). Asymptomatic infections occur often in females but infrequently in males.

The current methods for detection of C. *trachomatis* and/or *N. gonorrhoeae* include culture, immunoassays, nonamplified probes, and amplified probes (4, 6, 7). The development of amplified methods has demonstrated two advantages over non-amplified methods: increased sensitivity, and applicability to a variety of sample types. Historically, culture has been the "gold standard" for detection of C. *trachomatis.* However, the culture yield varies widely among laboratories, and culture in routine practice is less sensitive than amplified methods. Combining results from multiple methods of *C. trachomatis* detection improves accuracy for evaluating new tests in that infected and uninfected patients can be more reliably identified. For identification of *N. gonorrhoeae*, optimized culture methods continue to be the standard for diagnosing patients with gonococcal infections.

2. SAFETY PRECAUTIONS

3. 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.

4. 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 Tran 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.

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

Not applicable for this procedure.

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

Instrumentation

(1) GEN-PROBE® LEADER® HC+ Luminometer
(2) GEN-PROBE® Target Capture System (TCS)
(3) TECAN 71-705 NCS
(4) Multi-tube vortex mixer
(5) Circulating water bath

Other Materials

(1) APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens
(2) APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens
(3) APTIMA Vaginal Swab Specimen Collection Kit
(4) APTIMA Specimen Transfer Kit
(5) Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL)
(6) Micropipettor: 20 μL to 200 μL
(7) Tips, Pipetman P1000 Style, APTIMA Combo 2
(8) Tips, 1000 μL
(9) Pipette tips 20 μL to 200 μL
(10) Household bleach (sodium hypochlorite solution)
(11) APTIMA® Auto Detect Kit
(12) APTIMA® Controls Kit
(13) APTIMA® Penetrable Caps
(14) 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 45º 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 (15ºC to 30ºC) 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 62ºC 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 15ºC to 30ºC. 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 µ*L 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* µ*L 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.

APTIMA Combo 2 Selection Reagent
600 mM borate buffered solution containing surfactant.

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.

7. 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°C ± 1°C (for target capture, and primer annealing), a second water bath to 42°C ± 1°C (for amplification), and a third water bath to 62°C ± 1°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. Rack Setup

1. Allow the controls and specimens to reach room temperature prior to processing.
2. **Do not vortex specimens.**
3. Inspect transport tubes before piercing them:

    a. 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.

    b. 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.

    c. 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.

    d. If a urine specimen contains precipitates, heat the specimen at 37ºC 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.

4. In the Ten Tube Unit (TTU) rack, place enough TTUs to accommodate the controls and specimens.

5. Thoroughly mix the TCR plus TCR-B reagent. Using the repeat pipettor, add 100 ∝L into each reaction tube.

6. Hold the Positive Control, CT/Negative Control, GC tube in one hand or keep it in a rack. To work properly with the APTIMA Assay software, the Positive Control, CT/Negative Control, GC must be in the first position of the first TTU. This label is pink. The label text is “CONTROL + CT PCT / CONTROL – GC NGC”. Using a micropipettor, pierce the cap, taking care not to drive the tip into the bottom of the tube. Add 400 µL of the Positive Control, CT/Negative Control, GC to the first reaction tube. In the same manner, add 400 ∝L of the Positive Control, GC/Negative Control, CT to the second reaction tube. The label for the second control is blue-green. The label text is “CONTROL + GC PGC / CONTROL - CT NCT”.

7. Continue the rack setup procedure by adding 400 µL of each specimen to the remaining TTU tubes. Use a new pipette tip for each specimen and control. The acceptable volume of control or specimen added to the TTU is 400 µL ± 100 µL.

8. If specimens with standard (non-penetrable) caps are to be tested, they must be centrifuged for 5 minutes at 420 RCF to bring all of the liquid down to the bottom of the tube before uncapping. **Avoid splashing and cross-contamination.**

C. Target Capture

1. Cover the TTUs with sealing cards and shake the rack gently by hand. Do not vortex. Incubate the rack at 62°C ± 1°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.

D. Amplification

1. Using the repeat pipettor, add 75 μL 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 μL 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 62ºC ± 1ºC for 10 ± 5 minutes.
5. Transfer the rack into a water bath at 42ºC ± 1ºC for 5 ± 2 minutes.
6. With the rack in the water bath, carefully remove the sealing card and, using the repeat pipettor, add 25 μL 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 42ºC ± 1ºC for 60 ± 15 minutes.

E. 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 62ºC ± 1ºC 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 62ºC ± 1ºC for 10 ± 1 minutes.

c. Remove the rack from the water bath.

3. Detection

Detection must be performed at 18ºC to 28ºC.

a. Incubate the rack at 18ºC to 28ºC 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.

F. 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 and GC 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 and GC	1 to <85	85 to <250	250 to < 4,500
CT indeterminate	1 to <85	85 to <4,500	NA

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
GC indeterminate	1 to <85	85 to <4,500	NA

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.

The Positive Controls must produce the following test results:

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

1. The APTIMA Assay software automatically evaluates the controls according to the above criteria and will report the Run Status as PASS if the run control criteria are met, and FAIL if the run control criteria are not met.
2. If the Run Status is FAIL, all test results in the same run are invalid and must not be reported.
3. Specimens tested with a FAILED Run Status will be retested. A second consecutive FAILED Run Status will be recorded and reported to GenProbe for corrective actions.

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, 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, 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, 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, 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 Cytyc 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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