Laboratory Procedure Manual

Analyte: *Trichomonas vaginalis*

Matrix: **Urine**

Method: T. vaginal Using Gen-Probe APTIMA Combo on Panther System

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: TRICH_H & TRIC_H_R

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

Variable Name	SAS Label
URXUTRI	Trichomonas, urine

1. SUMMARY OF TEST PRINCIPLE

The GEN-PROBE APTIMA Trichomonas vaginalis 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 Trichomonas vaginalis (T. vaginalis) 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 TMA reaction replicates a specific region of the small ribosomal subunit from T. vaginalis via DNA and RNA intermediates and generates RNA amplicon molecules. Detection of the rRNA amplification product sequences is achieved using nucleic acid hybridization (HPA). A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with different acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA: DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA: DNA hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU).

2. SAFETY PRECAUTIONS

- For *in vitro* diagnostic use.
- 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, 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
    - 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.

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

A. Sample Information/Processing

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 μL to 200 μL
(3) Tips, Pipetman P1000 Style, APTIMA Combo 2
(4) Tips, 1000 μL
(5) Pipette tips 20 μL to 200 μL
(6) Household bleach (sodium hypochlorite solution)
(7) APTIMA® Auto Detect Kit
(8) APTIMA® Controls Kit
(9) APTIMA® Penetrable Caps
(10) Gloves

Reagents and Media

- Each APTIMA T.vaginalis Reagent Pack contains a refrigerated and non-refrigerated box:

  - **Refrigerated Box** (2°C to 8°C):
    - APTIMA T.vaginalis 2 Enzyme Reagent
      Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.
    - APTIMA T. vaginalis Amplification Reagent
      Nucleic acids dried in buffered solution containing < 5% bulking agent.
    - APTIMA T.vaginalis Probe Reagent
      Non-infectious chemiluminescent DNA probes (< 500 ng/vial) dried in succinate buffered solution containing < 5% detergent.
    - APTIMA T.vaginalis Target Capture Reagent B
      Non-infectious nucleic acid in a buffered solution containing < 5% detergent.
    - APTIMA Positive Control, T. vaginalis
      Non-infectious t. vaginalis nucleic acid in a buffered solution containing < 5% detergent.
    - APTIMA Negative Control, T. vaginalis
      Non-infectious non-target *nucleic acid in a buffered solution containing < 5% detergent.*

  - **Non-Refrigerated Box (15°C to 30°C):**
    - APTIMA T.vaginalis Target Capture Reagent
      Buffered salt solution containing solid phase (< 0.5 mg/ml) and capture oligomers.

  - APTIMA T.vaginalis Amplification Reconstitution Solution
    Aqueous solution containing preservatives.
  - APTIMA T. vaginalis Enzyme Reconstitution Solution
    HEPES buffered solution containing a surfactant and glycerol.
  - APTIMA T. vaginalis Probe Reconstitution Solution
    Succinate buffered solution containing < 5% detergent.
  - APTIMA T. vaginalis Selection Reagent
    600 mM borate buffered solution containing surfactant.
  - 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.

Supplies, Other Materials

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens
- APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens
- APTIMA Vaginal Swab Specimen Collection Kit
- Household bleach (sodium hypochlorite solution)
- APTIMA® Auto Detect Kit
- APTIMA® Controls Kit
- APTIMA® Penetrable Caps
- Gloves

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. Reagent Preparation

- To reconstitute the APTIMA T. vaginalis 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 45º 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 (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 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 62ºC 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.

B. Standards Preparation

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

C. Preparation of Quality Control Materials

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

D. 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 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.

- 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.

E. 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 worklist”.
- 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.

F. Method Performance Specifications

- Controls
  To work properly with the PANTHER APTIMA Assay software, one pair of controls is required. 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.

G. 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 T. vaginalis Assay as described in Section 6.
- Record environmental contamination.

H. 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 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”.
- 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.
- 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

I. 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

J. Interpretation of Test Results

Assay test results are automatically interpreted by the APTIMA Assay software, using the APTIMA T. vaginalis protocol. 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.

Test Interpretation	Total RLU (x1000) to give Tv Result		
Negative	0 to <100		
Positive	100 to <2400		
Invalid	above 2400		

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 and Negative Control act as controls for the **Target Capture**, **Amplification**, and **Detection** steps of the assay. 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)	T vaginalis Result	
Negative Control (NC)-TRICH	0 and <20	Negative	
Positive Control (PC)-TRICH	≥500 and <2400	Positive	

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 ay 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.

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.

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

Repeat run for individual sample.

12. LIMITATIONS OF THE PROCEDURE

Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given may result in erroneous results.
The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of *Trichomonas vaginalis*.
TV-positive mucoid samples may exhibit decreased RLU values. To ensure proper endocervical sampling, excess mucus should be removed.
Vaginal swab and PreservCyt Solution liquid Pap specimen sampling is not designed to replace cervical exams and endocervical specimens 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. Use of this assay is limited to personnel who have been trained in the procedure.
This method has been tested using only the following specimens:

- Patient-collected female and male urine specimens

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
Therapeutic failure or success cannot be determined with the APTIMA Trichomonas vaginalis Assay since nucleic acid may persist following appropriate antimicrobial therapy.
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.
Results from the APTIMA Trichomonas vaginalis Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
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.
A negative result does not preclude a possible infection because the presence of *Trichomonas tenax* or *Pentatrichomonas hominas* in a specimen may affect the ability to detect T.vaginalis rRNA.
The APTIMA Trichomonas vaginalis 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.
The APTIMA Trichomonas vaginalis Assay has not been validated for use with vaginal swab specimens collected by patients.
Performance has not been evaluated in pregnant women.
Performance has not been evaluated in women less than 14 years of age.
If a specimen has a small number of *T. vaginalis* organisms, uneven distribution of the trichomonas may occur, which may affect the ability to detect T. vaginalis rRNA in the collected material. If negative results do not fit the clinical impressions, a new specimen may be necessary.

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.

References

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