



## PACE® 2 CHLAMYDIA TRACHOMATIS

For *in vitro* diagnostic use.

### Intended Use

The GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the detection of *Chlamydia trachomatis* from endocervical, male urethral, and conjunctival swab specimens.

### Summary and Explanation of the Test

Chlamydiae are nonmotile, Gram negative, obligate intracellular bacteria that rely on the ATP produced by the host cell for replication. Chlamydial infections have been known for many years (10–12). *C. trachomatis* is responsible for approximately 50% of the cases of nongonococcal urethritis (7). *Chlamydia trachomatis* infections are one of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 1,030,911 new cases of *Chlamydia trachomatis* infections were reported in 2006 (4).

The *Chlamydia trachomatis* species is comprised of fifteen serovars that are responsible for the following diseases in humans: trachoma, inclusion conjunctivitis, lymphogranuloma venereum and other sexually transmitted diseases. The serovars D through K are the major cause of nongonococcal urethritis in men (13). Other clinical symptoms produced by *Chlamydia trachomatis* in humans include epididymitis, proctitis, cervicitis and acute salpingitis (7, 11, 14). In addition to the sexual transmission of chlamydial infections, newborn children are significantly at risk for inclusion conjunctivitis and chlamydial pneumonia from infected mothers (2, 5, 6, 15).

Historically, several methods for *Chlamydia trachomatis* detection have been utilized in the clinical laboratory, including cell culture, direct fluorescent antibody testing, and enzyme immunoassay. More recent methodologies for CT detection include direct DNA probe assays and nucleic acid amplification tests (NAATs). Cell culture was once considered to be the “gold standard” for detection of CT. Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe and NAATs.

The GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS uses the technique of nucleic acid hybridization (9) to identify *Chlamydia trachomatis* directly from endocervical, male urethral, and conjunctival swab specimens.

### Principles of the Procedure

Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes (9). The GEN-PROBE PACE 2 System uses a single-stranded DNA probe with a chemiluminescent label that is complementary to the ribosomal RNA of the target organism. After the ribosomal RNA is released from the organism, the labeled DNA probe combines with the target organism’s ribosomal RNA to form a stable DNA:RNA hybrid. The labeled DNA:RNA hybrid is separated from the non-hybridized probe and is measured in a GEN-PROBE LEADER luminometer. The test results are calculated as the difference between the response of the specimen and the mean response of the Negative Reference.

### Reagents

#### Materials Provided

The GEN-PROBE® PACE® 2 System for CHLAMYDIA TRACHOMATIS Kit

100 test kit (Cat. No. 201792; bioMérieux ref. 39211)

1000 test kit (U.S. and Canada only; Cat. No. 201792B)

Store at 2°C to 8°C upon receipt

Symbol	Component	Quantity		Description
		100 tests	1000 tests	
P	PACE 2 Chlamydia trachomatis Probe Reagent	2 x 6 mL when reconstituted	20 x 6 mL when reconstituted	Contains labeled, lyophilized <i>C. trachomatis</i> DNA probe in a buffer.
HB	PACE 2 Hybridization Buffer	2 x 6 mL	20 x 6 mL	Buffered solution.
S	PACE 2 Selection Reagent	1 x 100 mL	10 x 100 mL	Buffered solution.
SR	PACE 2 STD Separation Reagent	1 x 9 mL	10 x 9 mL	Solid phase in a buffered solution containing 0.02% sodium azide.
W	PACE 2 STD Wash Solution	3 x 200 mL	2 x 3800 mL	Buffered solution.
PCT	PACE 2 Chlamydia trachomatis Positive Control	1 x 3 mL	10 x 3 mL	Non-infectious <i>C. trachomatis</i> nucleic acid in a buffered solution.
NR	PACE 2 STD Negative Reference	1 x 7 mL	10 x 7 mL	Non-infectious nucleic acid in a buffered solution.
	Sealing cards	1 package	10 packages	

### Materials

**Note:** Materials available from Gen-Probe or your Gen-Probe distributor have catalog numbers listed.

#### Materials Required but Not Provided

GEN-PROBE® PACE® Specimen Collection Kits for Male Urethral or Conjunctival Specimens (Cat. No. 103275; bioMérieux ref. 39309) (50/box)

GEN-PROBE® PACE® Specimen Collection Kits for Endocervical Specimens (Cat. No. 103300; bioMérieux ref. 39301) (50/box)

PACE 2 Reaction Tubes (polystyrene 12 x 75 mm) (120/box, Cat. No. 102065; bioMérieux ref. 39307)

GEN-PROBE® Detection Reagent Kit (Cat. No. 201791; bioMérieux ref. 39300) (1200 tests)

GEN-PROBE® LEADER® Luminometer (Cat. No. 103100, 103100i-02/bioMérieux ref. 39400, 105194, 103200i)

GEN-PROBE® Magnetic Separation Unit (Cat. No. 101639 or equivalent; bioMérieux ref. 39306)

Vortex mixer

Covered water bath (60°C ± 1°C)

Micropipettes (100 µL)

Pipettes capable of delivering 1–25 mL

Lint-free wipes

### Optional Materials

GEN-PROBE® FAST Express Reagent Kit (Cat. No. 102930; bioMérieux ref. 39304)

GEN-PROBE® STD Proficiency Panel (Cat. No. 102325; bioMérieux ref. 39303)

GEN-PROBE® PACE®2 CHLAMYDIA TRACHOMATIS Probe Competition Assay Kit (Cat. No. 103548; bioMérieux ref. 39219)

PACE 2 Rapid Wash Station (Cat. No. 105641)

Bottle-Top Dispenser (1 to 2 mL, Cat. No. 101714; or 5 mL Cat. No. 103078)

Wash Bottle, 200 mL (Cat. No. 103919)

Electrostatic surface charge neutralizing device (ionizing blower) (Cat. No. 302481)

GEN-PROBE® Bottle Top Dispenser Adapter Kit (Cat. No. 104173)

### Warnings and Precautions

- A. For *in vitro* diagnostic use.
- B. This test system has been evaluated using endocervical, male urethral, and conjunctival swab specimens only.
- C. Separation Reagent MUST NOT freeze. The performance of the assay will be affected by use of improperly stored Separation Reagent. If the reagent has been frozen, the particles in the suspension may clump, resulting in a granular appearance that will not evenly disperse after thorough mixing. Visible clumps of Separation Reagent may adhere to the walls of the container. If this occurs, contact Gen-Probe Technical Support.
- D. Clean laboratory ware must be used to prepare reagents. Disposable polystyrene containers are strongly recommended.
- E. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- F. Specimens may be infectious. 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 type of diagnostic procedure.
  1. Thoroughly clean and disinfect all work surfaces.
  2. Autoclave any contaminated equipment or materials that have come in contact with the samples before discarding.
- G. Separation Reagent contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. Upon disposal of this reagent, always dilute the material with a large volume of water to prevent azide buildup in the plumbing.
- H. **WARNING: IRRITANTS, CORROSIVES.** Avoid contact of Detection Reagents I and II with skin, eyes and mucous membranes. Wash with water if these reagents come into contact with skin or eyes. If spills of these reagents occur, dilute with water before wiping dry. Rinse area and wipe dry.

- I. Do **NOT** interchange, mix or combine reagents from kits with different lot numbers except for STD Wash Solution.
- J. Expiration dates listed on the collection kits pertain to the collection site and not the testing facility. Samples collected any time prior to the expiration date of the collection kit, and transported and stored in accordance with the package insert, are valid for testing even if the expiration date on the collection tube has passed.

### Storage and Handling Requirements

Probe Reagent and Separation Reagent must be stored at 2°C to 8°C.

The Probe Reagent is stable for 3 weeks after reconstitution when stored at 2°C to 8°C.

The prepared Separation Suspension is stable for 6 hours after preparation when stored at 20°C to 25°C.

Other reagents contained in the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS are to be stored at 2°C to 25°C and are stable until the date stamped on the container.

**DO NOT FREEZE THE REAGENTS CONTAINED IN THIS KIT.**

### Specimen Collection and Preparation

The GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS is designed to detect the presence of *Chlamydia trachomatis* in endocervical, male urethral, and conjunctival specimens obtained from the urogenital tract or conjunctiva using the GEN-PROBE PACE Specimen Collection Kit.

Only swabs contained in the PACE Specimen Collection Kit can be used to collect patient specimens. The swabs collected from patients **MUST BE** transported to the laboratory in the GEN-PROBE transport medium.

- A. Collect swab samples as follows:
  1. Cervical swab specimens
    - a. Remove excess mucus from the cervical os and surrounding mucosa using one of the swabs provided in the cervical collection kit and discard the swab.
    - b. Insert the second swab from the collection kit into the endocervical canal.
    - c. Rotate the swab for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
    - d. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
    - e. Fully insert one swab into the GEN-PROBE transport tube.
    - f. Carefully snap the swab shaft at the scoreline to fit the tube; use care to avoid splashing of contents. **Cap the tube tightly.**
  2. Urethral swab specimens
    - a. Patient should not have urinated for at least 1 hour prior to sample collection.
    - b. Insert the swab from the urethral/conjunctival collection kit 2 to 4 cm into the urethra using a rotating motion to facilitate insertion.
    - c. Once inserted, rotate the swab gently using sufficient pressure to ensure the swab comes into contact with all urethral surfaces. Allow the swab to remain inserted for 2 to 3 seconds.
    - d. Withdraw the swab.
    - e. Fully insert the swab into the Gen-Probe transport tube.
    - f. Carefully snap the swab shaft at the scoreline to fit the tube; use care to avoid splashing of contents. **Cap the tube tightly.**

3. Conjunctival swab specimens
  - a. If pus or discharge is present, use a sterile, untreated dacron cleaning swab to clean the area.
  - b. Do not scrape the conjunctiva while cleaning the eye.
  - c. If both eyes are affected, first swab the least affected eye then swab the most affected eye.
  - d. Thoroughly swab the lower then the upper conjunctiva 2 to 3 times each using the male urethral/conjunctival swab provided.
  - e. Fully insert the swab into the GEN-PROBE transport tube.
  - f. Carefully snap the swab shaft at the scoreline to fit the tube; use care to avoid splashing of contents. **Cap the tube tightly.**
- B. Transport the tubes to the laboratory at 2°C to 25°C and store at 2°C to 25°C until tested. Samples should be assayed with the GEN-PROBE PACE 2 System within 7 days. If longer storage is necessary, process the specimen as described in *Sample Preparation* and freeze at -20°C to -70°C for up to 90 days after collection.
- C. During routine analysis, bloody specimens have not proven to interfere with assay performance. However, grossly bloody specimens (greater than 80 µL whole blood in 1 mL transport media) may interfere with performance.
- D. Specimens which require shipping should be transported to the laboratory in compliance with federal regulations covering transportation of etiological agents (HHS Publication No. CDC 93-8395). Store and test as described above.

## Test Procedure

- A. Sample Preparation
  1. Allow the specimens to reach room temperature prior to processing.
  2. Vortex each GEN-PROBE transport tube for at least 5 seconds.
  3. Express all liquid from the swab by pressing the swab against the wall of the tube. Discard the swab.
  4. Prior to testing, vortex the transport tube for at least 5 seconds to ensure homogeneity.
- B. Reagent Preparation
  1. All reagents EXCEPT the Probe Reagent, PACE 2 Hybridization Buffer, and Separation Reagent must reach room temperature prior to using. Probe Reagent and Separation Reagent must be maintained at 2°C to 8°C until used.
  2. Probe Reagent
 

**Lyophilized Probe**

If the PACE 2 Hybridization Buffer has formed a gel or has been stored at 2°C to 8°C, promptly vortex for 10 seconds upon removal. After vortexing, warm the reagent by swirling the vial in a water bath at 60°C ± 1°C for 3 to 4 minutes. Vortex again for 10 seconds to ensure a homogeneous solution. It may be necessary to repeat this procedure if the PACE 2 Hybridization Buffer is not homogeneous. Pipette 6.0 mL of PACE 2 Hybridization Buffer into lyophilized Probe Reagent. Allow the reagent to stand at room temperature for 2 minutes and then vortex for 10 seconds prior to use. Visually inspect to ensure that the reagent is completely rehydrated and homogeneous. Record on the label the date reconstituted.

### Reconstituted Probe

The reconstituted Probe Reagent is stable for 3 weeks when stored at 2°C to 8°C or until the date stamped on the reagent container, whichever comes first. If the reconstituted Probe Reagent has been refrigerated, vortex for 10 seconds then warm it by swirling the vial in a water bath at 60°C ± 1°C for 2 minutes. Prior to use, vortex again for 10 seconds to ensure homogeneity. It may be necessary to repeat this procedure if the reconstituted Probe Reagent is not homogeneous.

### 3. Separation Suspension

Determine the number of tests to be performed. Calculate the volumes of Selection Reagent and Separation Reagent as follows:

Volume of Selection Reagent (mL)

- = number of tests + 2 extra tests (with eppendorf repeating pipettor)
- = number of tests + 10 extra tests (with bottle top dispenser)

Volume of Separation Reagent (mL)

$$= \frac{\text{Volume of Selection Reagent (mL)}}{20}$$

Pour the required volume of Selection Reagent into a clean dry container. Mix the Separation Reagent, add the required volume to the Selection Reagent, and mix well. Prepared Separation Suspension is stored at room temperature and is stable for 6 hours.

Separation Suspension Preparation (Example):

8 tests + 2 extra for eppendorf pipettor = 10 tests

Number of tests	Selection Reagent	Separation Reagent
8 + 2	10 mL	0.5 mL
18 + 2	20 mL	1.0 mL
48 + 2	50 mL	2.5 mL
98 + 2	100 mL	5.0 mL

- C. Hybridization
  1. Label tubes with sample identification numbers. Include three tubes for the Negative Reference and one for the Positive Control. Label near the tops of the tubes only.
  2. Insert the tubes into the tube rack of the GEN-PROBE Magnetic Separation Unit. Set aside the base portion of the separation unit for later use.
  3. Vortex each specimen for 5 seconds.
  4. Pipette 100 µL of each of the controls and specimens to the bottom of the respective tubes.
  5. Pipette 100 µL of the Probe Reagent to the BOTTOM of each tube, taking care not to touch the top or sides of the tube.
  6. Cover the tubes with Sealing Cards ensuring that each tube is sealed.
  7. Shake the rack 3 to 5 times to mix.
  8. Incubate the tubes in a water bath at 60°C ± 1°C for 1 hour. Do **NOT** place the magnetic separation unit base in the water bath.
- D. Equipment Preparation
  1. Prepare the GEN-PROBE LEADER luminometer for operation. Make sure there are sufficient volumes of Detection Reagents I and II to complete the tests.

## E. Separation

1. Remove the tube rack from the water bath and remove the Sealing Cards.
2. Pipette 1 mL of the well-mixed, prepared Separation Suspension into each tube.
3. Cover the tubes with Sealing Cards and vigorously shake the tube rack 3 to 5 times to mix. A foam head should be present in each tube.
4. Immediately incubate the tubes in a water bath at  $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 10 minutes.
5. Remove the tube rack from the water bath. Remove the Sealing Cards and place the tube rack on the base of the Magnetic Separation Unit for 5 minutes at room temperature.
6. Holding the tube rack and base of the GEN-PROBE Magnetic Separation Unit together, decant the supernatants. Before turning tubes upright, shake the unit 2 to 3 times and then blot tubes 3 times for 5 seconds each on absorbent paper.
7. DO NOT REMOVE THE TUBE RACK FROM THE GEN-PROBE MAGNETIC SEPARATION BASE. Fill each tube to the rim with Wash Solution. See *Procedural Notes* regarding Wash Solution addition.
8. Allow the tubes to remain on the magnetic separation base for 20 minutes at room temperature.
9. Holding the tube rack and base together, decant supernatants. Before turning tubes upright, shake the unit 2 to 3 times. DO NOT BLOT. Approximately 50 – 100  $\mu\text{L}$  of Wash Solution should remain in each tube.
10. Separate the tube rack from the base and shake the tube rack to resuspend the pellets.

## F. Detection

1. Select the appropriate protocol from the LEADER luminometer software.
2. Use a deionized water-saturated, lint-free wipe and wipe each tube 1 or 2 times to reduce static charge and to ensure that no residue is present on the outside of the tube. Re-wet the lint-free wipe after 30 tubes or if it seems to be drying. An electrostatic surface charge neutralizing device can be used in conjunction with wet wiping in dry locations. Contact Gen-Probe Technical Support for more information.
3. Ensure that the pellets are resuspended and insert the tubes into the LEADER luminometer according to the prompts provided by the instrument software.
4. Read the tubes in the following order:
  - a. Negative Reference, 3 tubes
  - b. Positive Control, 1 tube
  - c. Specimen tubes
5. When the analysis is complete, remove the tube(s) from the LEADER luminometer.

## Procedural Notes

## A. PACE 2 Hybridization Buffer and Probe Reagent

Gel formation of the PACE 2 Hybridization Buffer and reconstituted Probe Reagent may occasionally occur. Vortexing, heating and swirling of reagents at  $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$  is imperative to minimize gel formation and ensure a homogeneous solution.

## B. Specimens

Occasionally a specimen may be too viscous to pipet. Be sure that specimens are at room temperature and vortex to liquefy. The GEN-PROBE FAST Express reagent may be used to simplify specimen preparation.

## C. Pipetting

For convenience, repeating pipettors or dispensers may be used for addition of Probe Solution, Separation Suspension, and Wash Solution. Pipettors with disposable tips are recommended for pipetting specimens and controls to avoid sample carry-over and cross-contamination. Care should be taken to pipette Probe Reagent to the BOTTOM of tubes without inserting the pipette tip into the tubes or touching the tip to the rim of each tube. When adding the reagents, angle the solutions toward the front sides of the tubes, not straight to the bottoms, to avoid splashback.

## D. Blotting

Discard absorbent paper after each blotting to avoid contamination. DO NOT BLOT AFTER THE WASH STEP.

## E. Temperature

The hybridization and separation reactions are temperature dependent. Therefore, it is imperative that the water bath and reaction tubes be equilibrated uniformly during these steps. A covered water bath capable of maintaining  $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$  should be used.

## F. Washing

The Wash Solution should be injected into each tube using only enough force to obtain a 1-cm foam head. Angle the Wash Solution toward the front sides (or back sides) of the tubes, not to the left or right sides or straight to the bottoms, to avoid directly hitting the magnetic particle pellet with the Wash Solution stream and to avoid splashback. After adding Wash Solution to all tubes in the rack, care should be taken to go back and "top off" each tube. Some, not all, of the foam may remain. Failure to deliver Wash Solution in the specified manner may result in spurious results.

If using the 1 – 2 mL bottle-top dispenser or 5 mL bottle-top dispenser:

- a. Set the dispenser at 2 mL.
- b. Add two 2 mL additions of Wash Solution into each tube with enough force to obtain a 1-cm foam head.
- c. Slowly add one 1 – 2 mL addition of Wash Solution into each tube to top off with minimal overflow. Excessive force should not be used to top off the liquid in each tube.

If using the Wash Bottle Cap Assembly:

- a. Add approximately 4 mL of Wash Solution into each tube (only fill below or up to the rim of each tube on initial addition).
- b. Slowly add approximately 1 to 2 mL into each tube to top off with minimal overflow. Excessive force should not be used to top off the liquid in each tube.

**Note:** The Wash Bottle Cap Assembly is an optional method for delivering Wash Solution. Each laboratory should validate that this assembly yields assay performance equivalent to that of their current validated method of Wash Solution addition. Prior to using a new wash bottle and cap assembly, pour wash into the bottle. Screw cap onto bottle. Discard the first 5 mL by squirting through the cap.

If using the GEN-PROBE PACE 2 Rapid Wash Station, follow directions in the GEN-PROBE PACE 2 Rapid Wash Station package insert up to the "Wash Procedure."

- a. Set the volume of the Dispense Pump to 40 mL.
- b. Prime as directed in the Rapid Wash Station package insert.
- c. For the first addition of Wash Solution, use only enough force to obtain a 1-cm foam head.

- d. For the second addition of Wash Solution, change the dispense setting to 14 mL as directed in the Rapid Wash Station package insert, and add Wash Solution slowly to avoid splashback.

G. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened reagents or reaction tubes. Gen-Probe recommends that customers experiencing difficulty with the test avoid using this type of laboratory glove. Using powderless gloves (no talcum powder) will avoid this difficulty.

H. Detection

Tubes should be read in the LEADER luminometer within 60 minutes of decanting the Wash Solution. Tubes should be maintained at 20°C to 25°C prior to reading.

## Test Interpretation – QC/Patient Results

A. Calculation of Results

The results of the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS are calculated based on the difference between the response in Relative Light Units (RLU) of the specimen and the mean of the Negative Reference.

Mean of the Negative Reference = Sum of the three Negative Reference replicates divided by 3.

Example:

$$\text{Mean of the Negative Reference} = \frac{(65 \text{ RLU} + 71 \text{ RLU} + 80 \text{ RLU})}{3} = 72 \text{ RLU}$$

$$\text{Specimen Response} = 900 \text{ RLU}$$

$$\text{Difference} = 900 \text{ RLU} - 72 \text{ RLU} = 828 \text{ RLU} \quad (\text{Positive})$$

The LEADER luminometer prints the specimen response and compares this response to the calculated assay cutoff. A positive or negative interpretation as compared to this cutoff is printed. See the Operator's Manual for detailed protocols.

B. Interpretation of Results<sup>1</sup>

POSITIVE — The difference is  $\geq 350$  RLU.

NEGATIVE — The difference is  $< 350$  RLU.

A positive result indicates that *Chlamydia trachomatis* is present in the specimen tested and strongly supports a diagnosis of chlamydial infection.

A negative result indicates the absence of *Chlamydia trachomatis* in the specimen tested.

C. Quality Control and Acceptability of Results

Negative Reference:

The response of each Negative Reference value should be  $\leq 200$  RLU. All Negative Reference values should fall within 30% of the mean response for the Negative Reference (i.e., the Coefficient of Variation should be  $\leq 30\%$ ). If one value falls outside these ranges or is invalidated by a high background error, it may be deleted from the calculations by following the instructions in the LEADER luminometer Operator's Manual. If two values fall outside these ranges, the test should be repeated. If this is a frequent

occurrence, re-evaluate the technique used and contact Gen-Probe Technical Support if the problem persists.

Positive Control:

The difference between the response of the Positive Control and the mean response of the Negative Reference should be  $> 600$  RLU. If the Positive Control value repeatedly falls out of specification contact Gen-Probe Technical Support.

If the Positive Control or Negative Reference values are not in the required range, the test result must not be reported.

D. Additional Procedure (Optional)

The PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay (PCA) can be used in conjunction with the PACE 2 System for CHLAMYDIA TRACHOMATIS as a supplemental test to detect nonspecific signal in endocervical and male urethral swab specimens. Specimens are first tested in the PACE 2 assay to differentiate positive from negative specimens. Positive specimens can then be tested in the PCA assay. Consult the PCA package insert or the 1993 CDC guidelines for information on samples that should be retested (3).

## Limitations

- A. This method has been tested using endocervical, male urethral, and conjunctival swab specimens only. Performance with other specimens has not been assessed. A negative test result does not exclude the possibility of infection because test results may be affected by improper specimen collection, technical error, specimen mixup, concurrent antibiotic therapy, or the concentration of organisms in the specimen may be below the sensitivity of the test. Proper training of personnel collecting the swab specimens is important so as to reduce the possibility of negative results due to improper sample collection. Results from the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- B. During routine analysis, bloody specimens have not proven to interfere with assay performance. However, grossly bloody specimens (greater than 80  $\mu$ L whole blood in 1 mL transport media) may interfere with performance.
- C. The PACE 2 assay has been evaluated for interference by gynecological lubricants and spermicides. The data indicate that in normal usage no interference will be observed. For additional information on particular products, contact Gen-Probe Technical Support.
- D. All *Chlamydia trachomatis* identification methods can yield false positive results. In those circumstances where diagnosis could lead to adverse psychosocial impacts, additional testing methods are recommended. Culture is the only recommended procedure for diagnosing chlamydial infection in cases of suspected child abuse.
- E. As in any clinical situation, diagnosis should not be based on the results of a single laboratory test. If the test result is negative and the clinical indications strongly suggest chlamydial infection, additional specimens should be collected for further testing.
- F. As in any disease state, the positive predictive value of this assay will decrease as the prevalence decreases in the population.

## Clinical Performance Characteristics

A. Urethral and Endocervical Specimens

The GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS was compared to culture methods using 1592 urogenital specimens where the overall positivity rate was 9.9%. The specimens were categorized as either positive (difference  $\geq 350$  RLU) or negative (difference  $< 350$  RLU). A comparison of these results to standard culture methods using cycloheximide

<sup>1</sup> Note. A small fraction of samples may yield results between 200 and 350 RLU. As required in France by the *Agence du Médicament* these samples need to be tested in the PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay (PCA). The performance of the PCA-CT in identifying true positive specimens below the assay cut-off (range to be retested) was discussed in the study by Beebe et al. (1).

treated McCoy cells stained with fluorescein-labeled monoclonal antibodies is shown below.

1. Performance Summary with Initial Data

Low Positivity Rate (< 8%)

PACE 2 / Culture						
PACE 2	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
Culture	Pos	Neg	Pos	Neg		
<b>Population</b>						
Women (4.1%)	31	2	2	763	93.9%/99.7%	99.5%

High Positivity Rate (> 8%)

PACE 2 / Culture						
PACE 2	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
Culture	Pos	Neg	Pos	Neg		
<b>Population</b>						
Men (13.9%)	4	0	1	31	80.0%/100.0%	97.2%
Women (15.7%)	109	19	10	620	91.6%/97.0%	96.2%

Combined High and Low Positivity Rate

PACE 2 / Culture						
PACE 2	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
Culture	Pos	Neg	Pos	Neg		
<b>Population</b>						
Men (13.9%)	4	0	1	31	80.0%/100.0%	97.2%
Women (9.8%)	140	21	12	1383	92.1%/98.5%	97.9%
<b>Total (9.9%)</b>	<b>144</b>	<b>21</b>	<b>13</b>	<b>1414</b>	<b>91.7%/98.5%</b>	<b>97.9%</b>

The positive and negative predictive values for various positivity rates at a sensitivity and specificity of 91.7% and 98.5% are shown below.

Predictive Values

	POSITIVITY RATE			
	5%	10%	15%	20%
<b>Positive Predictive Value (%)</b>	76.1	87.4	91.5	93.9
<b>Negative Predictive Value (%)</b>	99.6	99.1	98.5	97.9

These results agree with reports indicating a 5% to 13% discrepancy rate as a result of sample variation using standard culture techniques.

2. Performance Summary with Discrepant Analysis

Seventeen of the 21 specimens producing an "Apparent False Positive Result" were tested for the presence of *Chlamydia* specific nucleic acid using other DNA probe techniques. All of the 17 specimens tested contained *Chlamydia* specific nucleic acid. Additionally, 6 of the 17 specimens tested were positive using an enzyme immunoassay for *C. trachomatis*. The results are provided in the following tables.

Low Positivity Rate (< 8%)

PACE 2 / Culture						
PACE 2	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
Culture	Pos	Neg	Pos	Neg		
<b>Population</b>						
Women (4.1%)	33	0	2	763	94.3%/100.0%	99.8%

High Positivity Rate (> 8%)

PACE 2 / Culture						
PACE 2	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
Culture	Pos	Neg	Pos	Neg		
<b>Population</b>						
Men (13.9%)	4	0	1	31	80.0%/100.0%	97.2%
Women (17.8%)	125	3	10	620	92.5%/99.5%	98.3%

Combined High and Low Positivity Rate

PACE 2 / Culture						
PACE 2	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
Culture	Pos	Neg	Pos	Neg		
<b>Population</b>						
Men (13.9%)	4	0	1	31	80.0%/100.0%	97.2%
Women (10.8%)	158	3	12	1383	92.9%/99.8%	99.0%
<b>Total (10.9%)</b>	<b>162</b>	<b>3</b>	<b>13</b>	<b>1414</b>	<b>92.6%/99.8%</b>	<b>99.0%</b>

The positive and negative predictive values for various positivity rates at a sensitivity and specificity of 92.6% and 99.8% are shown below.

Predictive Values

	POSITIVITY RATE			
	5%	10%	15%	20%
<b>Positive Predictive Value (%)</b>	96.1	98.0	98.7	98.9
<b>Negative Predictive Value (%)</b>	99.6	99.2	98.7	98.1

B. Conjunctival Specimens

The GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS was compared to culture methods with direct fluorescent antibody (DFA) staining using 325 conjunctival swab specimens collected from symptomatic neonates, infants, and adults. The overall positivity rate was 8.3%. The specimens were categorized as either positive ( $\geq 350$  RLU) or negative ( $< 350$  RLU). A comparison of these units to standard culture methods using fluorescein-labeled monoclonal antibodies is shown below.

1. Performance Summary with Initial Data

PACE 2 / Culture with DFA						
PACE 2 DFA	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
<b>Population</b>						
Total (8.0%)	32	4	1	377	97.0%/99.0%	98.8

2. Performance Summary with Discrepant Analysis

PACE 2 / Culture with DFA						
PACE 2 DFA	Pos	Pos	Neg	Neg	Sensitivity/ Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
<b>Population</b>						
Total (8.2%)	33	3*	1	377	97.1%/99.2%	99.0

\*One specimen subsequently resolved as culture positive.

Analytical Performance Characteristics

A. Within-Run Precision

1. Urethral and Endocervical Specimens

The within-run precision of the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS was calculated by assaying three concentrations of *Chlamydia trachomatis* ribosomal RNA using 10 replicates in a single assay.

Sample	A	B	C
Number of Replicates	10	10	10
Mean Response (RLU)	932.2	3231.1	6207.8
Standard Deviation (RLU)	27.5	101.5	89.8
Coefficient of Variation	3.0%	3.1%	1.4%

2. Conjunctival Specimens

The within-run precision of the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS was calculated by assaying three concentrations of *Chlamydia trachomatis* ribosomal RNA using 10 replicates in a single assay.

Sample	A	B	C
Number of Replicates	10	10	10
Mean Response (RLU)	623	1144	5675
Standard Deviation (RLU)	24	38	197
Coefficient of Variation	3.8%	3.3%	3.5%

B. Between-Run Precision

1. Urethral and Endocervical Specimens

Between-run precision was calculated by assaying the same three concentrations of *Chlamydia trachomatis* ribosomal RNA using single determinations in 12 consecutive runs.

Sample	A	B	C
Number of Replicates	12	12	12
Mean Response (RLU)	902.9	3303.1	6257.9
Standard Deviation (RLU)	51.7	164.5	251.4
Coefficient of Variation	5.7%	5.0%	4.0%

2. Conjunctival Specimens

Between-run precision was calculated by assaying the same three concentrations of *Chlamydia trachomatis* ribosomal RNA on each of two consecutive days.

Sample	A	B	C
Number of Replicates	10	10	10
Mean Response (RLU)	588	1099	5391
Standard Deviation (RLU)	49	64	402
Coefficient of Variation	8.3%	5.8%	7.5%

C. Analytical Sensitivity—Urethral and Endocervical Specimens

The analytical sensitivity (limits of detection) of the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS was determined for urethral and endocervical samples by directly comparing dilutions of freshly grown *Chlamydia trachomatis* in cell culture and in the PACE 2 assay. The sensitivities for the 15 *C. trachomatis* serovars at the assay cut-off of 350 Net RLU ranged from 24 to 2,332 inclusion-forming units (IFU)/assay. The sensitivities (IFU/assay) for the serovars most frequently associated with urogenital and conjunctival disease were: Serovar D, 577; Serovar E, 745; Serovar F, 1607; Serovar G, 418; Serovar H, 565; Serovar I, 128; Serovar J, 239; Serovar K, 2042.

D. Analytical Specificity

1. Urethral and Endocervical Specimens

A total of 66 culture isolates were evaluated using the *Chlamydia trachomatis* probe. These isolates included 17 organisms that may be isolated from the urogenital tract and 27 additional organisms that represent a phylogenetic cross-section of organisms. Culture isolates of 15 *Chlamydia trachomatis* serovars, 4 strains of *Chlamydia psittaci* and 3 isolates of *Chlamydia pneumoniae* (TWAR) were also tested. Only the 15 *Chlamydia trachomatis* serovars produced a positive result in the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS.

2. Conjunctival Specimens

A total of 41 culture isolates were evaluated using the *Chlamydia trachomatis* probe. These isolates included 22 organisms that may be associated with eye disease and 17 additional organisms that represent a phylogenetic cross-section of organisms. *Chlamydia psittaci* and *Chlamydia pneumoniae* (TWAR) were also tested and, like the other organisms tested, yielded no cross-reactions.

E. Recovery

1. Urethral and Endocervical Specimens

*Escherichia coli*, *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, and *Candida albicans* each were added at concentrations up to 10 million cells per test to samples containing between 1 and 50 chlamydial inclusion forming units per test. These additions did not interfere with the recovery of *Chlamydia trachomatis* using the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS.

2. Conjunctival Specimens

Ribosomal RNA isolated from *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Chlamydia pneumoniae* was added at either 0.1 µg/assay or 1.0 µg/assay to samples containing different concentrations of *Chlamydia trachomatis* ribosomal RNA. These additions did not interfere with the recovery of *C. trachomatis* RNA using the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS.

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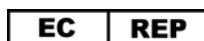
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