



# PACE<sup>®</sup> 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay

For *in vitro* diagnostic use.

## Intended Use

The PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay (PCA) is a rapid DNA probe test that uses the technique of competitive nucleic acid hybridization. PCA can be used in conjunction with the PACE 2 assay as a supplemental test to detect nonspecific signal in endocervical and male urethral specimens that test positive in the PACE 2 System for CHLAMYDIA TRACHOMATIS.

## Summary and Explanation of the Test

*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 (6). *Chlamydiae* are nonmotile, Gram negative, obligate intracellular bacteria that rely on the ATP produced by the host cell for replication.

*C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and pelvic inflammatory disease (3, 9, 12, 13, 14). Children born to infected mothers are at significantly higher risk for inclusion conjunctivitis and chlamydial pneumonia (2, 7, 8, 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 to identify *C. trachomatis* directly from endocervical, male urethral, and conjunctival swab specimens. This method uses a chemiluminescent, single-stranded DNA probe that is complementary to the ribosomal RNA of the target organism (11). After the ribosomal RNA is released from the organism, the labeled DNA probe combines with it to form a stable DNA:RNA hybrid. The presence of stable DNA:RNA hybrids is detected in a GEN-PROBE LEADER luminometer by virtue of their chemiluminescent labels.

According to 1993 Centers for Disease Control (CDC) guidelines (5), positive test results obtained for persons "who are at low risk for infection or for whom a misdiagnosis of chlamydial infection could lead to social/psychological distress" should be verified. Consult the referenced guidelines for further information. 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. Naturally occurring specimens that yield nonspecific signals are rare, but do occur. The causes and mechanisms of nonspecific signals in these specimens are not known. It is known, however, that extrinsic interference can occur under unique conditions.

## Principles of the Procedure

The PCA assay involves two reactions. The first is a repeat of the PACE 2 assay using the PACE 2 DNA probe with a chemiluminescent label. The second reaction in the PCA assay employs a tube containing excess lyophilized probe reagent that is identical to the PACE 2 probe reagent except that it is lacking the chemiluminescent label. Standard probe with a chemiluminescent label is added to both the PCA reaction tubes. Because the labeled and unlabeled probe reagents are both complementary to the rRNA of the target organism, they will compete with one another to form a stable DNA:RNA hybrid with the target. Replacement of labeled probe by an unlabeled probe in the DNA:RNA hybrid results in a decrease in detectable signal in the assay.

A reduction of 70% or greater in the signal generated in the PCA reaction tube containing unlabeled probe as compared to the signal generated in the tube containing only labeled probe indicates that the specimen contains *C. trachomatis* and is not giving a positive reaction because of nonspecific signal.

## Reagents

Reagents for the PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay are provided in three separate reagent kits. Also see *Materials Required But Not Provided*.

## Materials Provided

**PACE<sup>®</sup> 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay Kit (Cat. No. 103548, 103548F-01/bioMérieux ref. 39219)**

2°C to 8°C

Symbol	Component	Quantity	Description
PRCR	PACE 2 Probe Competition Reagent - <i>Chlamydia trachomatis</i>	2 x 10 tubes 20 tests	Lyophilized, unlabeled <i>C. trachomatis</i> DNA probe.

## Materials

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

## Materials Required But Not Provided

- GEN-PROBE<sup>®</sup> PACE<sup>®</sup> 2 System for CHLAMYDIA TRACHOMATIS (Cat. No. 201792/bioMérieux ref. 39211, 201792B)
- GEN-PROBE<sup>®</sup> Detection Reagent Kit (Cat No. 201791/bioMérieux ref. 39300) (1200 tests)
- GEN-PROBE<sup>®</sup> PACE<sup>®</sup> Specimen Collection Kits for Male Urethral or Conjunctival Specimens (Cat. No. 103275/bioMérieux ref. 39309) (50/box)
- GEN-PROBE<sup>®</sup> PACE<sup>®</sup> Specimen Collection Kits for Endocervical Specimens (Cat. No. 103300/bioMérieux ref. 39301) (50/box)
- PACE 2 Reaction Tubes (Cat. No. 102065/bioMérieux ref. 39307)
- GEN-PROBE<sup>®</sup> LEADER<sup>®</sup> Luminometer
- GEN-PROBE<sup>®</sup> 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)  
 PACE 2 Rapid Wash Station (Cat. No. 105641)  
 Bottle-Top Dispenser (1 to 2 mL, Cat. No. 101714; or 5 mL Cat.  
 No. 103078)  
 Bottle-Top Adapter Kit (Cat. No. 104173)  
 Wash Bottle, 200 mL (Cat. No. 103919)  
 Electrostatic surface charge neutralizing device (ionizing blower)  
 (Cat. No. 302481)

### Warnings and Precautions

- A. For *in vitro* diagnostic use.
- B. Use universal precautions when performing this assay (4).
- C. This test system has been evaluated using endocervical and male urethral swab specimens collected with the GEN-PROBE PACE Specimen Collection Kit only.
- D. Use only supplied or specified disposable laboratory ware.
- E. Reagents in this kit contain 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.
- F. **WARNING: CORROSIVE PRODUCT:** 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.
- G. Do not interchange, mix, or combine reagents from kits with different lot numbers except for STD Wash Solution.
- H. 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 Competition Reagent Tubes must be stored at 2°C to 8°C. The Probe Competition Reagent Tubes are stable in the unopened pouches until the expiration date indicated. Once opened, the pouch should be resealed and the tubes should be used within 2 months and prior to the expiration date.

### Specimen Collection and Preparation

The GEN-PROBE PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay is designed to detect nonspecific signal in specimens obtained from the male urethra and the female endocervical canal using the GEN-PROBE PACE 2 Specimen Collection Kit. Samples tested in PCA are those that have been processed and tested in the PACE 2 System for CHLAMYDIA TRACHOMATIS and shown to be positive.

- A. Only specimens collected and processed in accordance with the directions outlined in the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS package insert may be tested in the PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay.

All specimens must be collected using a GEN-PROBE PACE Specimen Collection Kit.

- B. Specimens should be stored at 2°C to 25°C until they are tested. Specimens should be assayed within 7 days. If longer storage is necessary, freeze the samples 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 medium) 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. Equipment Preparation
 

Prepare the GEN-PROBE LEADER luminometer for operation. Make sure there are sufficient volumes of Detection Reagents I and II to complete the tests.
- B. Specimens/Controls
 

For each specimen to be tested, one standard PACE 2 reaction tube and one Probe Competition Assay (PCA) tube, which is capped and from the pouch, and contains lyophilized PCA *C. trachomatis* (CT) probe, will be needed. One set of PCA Positive Controls will be run with each set of specimens tested. The PCA Positive Controls will consist of one standard PACE 2 tube (A tube) and one PCA tube containing lyophilized CT probe (B tube). As well, three standard PACE 2 Negative References will be run in each assay rack using standard PACE 2 reaction tubes. The Positive Controls are used to indicate that the assay has been run correctly and that the competition reaction is functioning properly. The Negative References provide a measure of the assay background and are used to calculate the run cut-off.

**Note:** No control for nonspecific signal (i.e., no competition) is provided.
- C. Sample Preparation
 

All samples tested in the PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay must be run in both the standard PACE 2 and PCA reaction tubes in the same run. There must be sufficient sample volume to complete both reactions.

  1. Open the foil pouch by cutting evenly across the top of the pouch. Remove enough Probe Competition Reagent Tubes to test the urogenital specimens and controls. Reseal the pouch by folding the opened edge over several times and securing with adhesive tape or a clip. Leave the desiccant pillow in the pouch.
  2. Remove the specimens to be tested from the freezer or refrigerator. Allow the specimens to reach room temperature prior to testing.
  3. Set up tubes in a magnetic rack for the PCA testing in the following order:
    - a. **Negative Reference:** Three standard PACE 2 reaction tubes. Label each of these tubes "Negative".
    - b. **PCA Positive Control:** One standard PACE 2 reaction tube and one capped PCA tube from the PCA C. TRACHOMATIS pouch. Label the standard PACE 2 reaction tube "PCA Positive A" and label the capped PCA tube from the pouch "PCA Positive B".
    - c. **Specimens:** One standard PACE 2 reaction tube and one capped PCA tube from the PCA C. TRACHOMATIS pouch for each specimen to be tested. Label each set of

specimens with the specimen identification number followed by an "A" for the standard PACE 2 reaction tube or a "B" for the capped PCA tube, respectively.

4. To each of the three tubes labeled "Negative," add 100  $\mu$ L of PACE 2 Negative Reference according to the directions in the PACE 2 System for CHLAMYDIA TRACHOMATIS package insert.
  5. To the two PCA Positive Control tubes labeled "A" and "B," add 100  $\mu$ L of PACE 2 *C. trachomatis* Positive Control according to the directions in the PACE 2 System for CHLAMYDIA TRACHOMATIS package insert.
  6. Vortex each sample for at least 5 seconds.
  7. For each sample to be tested, add 100  $\mu$ L of sample to the "A" reaction tube (standard PACE 2 reaction tube) and 100  $\mu$ L to the "B" reaction tube (capped PCA tube containing lyophilized probe).
- D. Hybridization
1. Reconstitute the PACE 2 Probe Reagent as described in the PACE 2 System for CHLAMYDIA TRACHOMATIS package insert.
  2. Pipette 100  $\mu$ L of the PACE 2 Probe Reagent to the BOTTOM of every tube in the rack, taking care not to touch the top or sides of the tube.
  3. Cover the tubes tightly with Sealing Cards. Shake the rack to mix.
  4. Proceed with the PACE 2 assay as described in the PACE 2 System for CHLAMYDIA TRACHOMATIS package insert.
- E. 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 in the LEADER luminometer according to the prompts provided by the instrument software.
  4. Read the tubes in the following order:
    - a. 3 negative references
    - b. 2 PCA Positive Controls ("A" tube then "B" tube)
    - c. 2 sample tubes ("A" tube then "B" tube).

Each set of sample tubes should be identified with an "A" or a "B" for the first and second tube, respectively.
  5. When the analysis is complete, remove the tubes from the LEADER luminometer.
- 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.
- 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°C  $\pm$  1°C should be used.
- F. Wash Solution Addition
- 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 reagent 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

## Procedural Notes

### A. 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°C  $\pm$  1°C is imperative to minimize gel formation and ensure a homogeneous solution.

### B. Specimens

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

Station package insert, and add Wash Solution slowly to avoid splashback.

F. 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.

G. 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 CHLAMYDIA TRACHOMATIS Probe Competition Assay are calculated based on the difference between the response in Relative Light Units (RLU) of the sample in Tube B compared to that of the same sample in Tube A. A value of 350 RLU or greater over the mean negative reference is required in Tube A. Samples yielding less than 350 RLU over the mean negative reference are negative for *C. trachomatis* and, therefore, the percent competition value should not be evaluated.

Signal is measured as net signal following subtraction of the mean of the Negative Reference.

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

Example:

$$\begin{aligned} \text{Mean of the Negative Reference} &= \frac{(55 \text{ RLU} + 60 \text{ RLU} + 50 \text{ RLU})}{3} \\ &= 55 \text{ RLU} \end{aligned}$$

Specimen Response Tube A = 894 RLU

Net Signal = 894 - 55 = 839 RLU

Specimen Response Tube B = 60 RLU

$$\begin{aligned} \text{Percent Competition} &= \frac{(\text{Signal Tube A} - \text{Signal Tube B})}{(\text{Net Signal Tube A})} \\ &= \frac{(894 - 60)}{(839)} \times 100 \\ &= 99.4\% \end{aligned}$$

The LEADER luminometer prints the sample responses for Tubes A and B. For Tube A, the LEADER luminometer software compares the value to an assigned cut-off and prints a positive or negative interpretation. For Tube B, the LEADER luminometer software calculates the difference between the Tube A and Tube B values, compares the difference value to an assigned assay cut-off, and prints a true or false interpretation. See the Operator's Manual for detailed protocol.

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

True = Tube A ≥ 350 Net RLU and  
% Competition ≥ 70%

False = Tube A ≥ 350 Net RLU and  
% Competition < 70%

A true result indicates that the signal obtained using the PACE 2 System for CHLAMYDIA TRACHOMATIS is the result of the presence of *C. trachomatis* in the sample.

A false result indicates that the PACE 2 signal is due to interfering material in the sample and not to the presence of *C. trachomatis*.

A result of B > A could be caused by technical error, including reversal of tube order, or assay variability. Each laboratory should establish their own performance guidelines.

C. Quality Control and Acceptability of Results

Negative Reference

The response of each Negative Reference value should be ≤ 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 ≤ 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 call Gen-Probe Technical Service 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 greater than 600 RLU for Tube A. The percent competition must be greater than or equal to 90%. If the Positive Control value repeatedly falls out of specification contact Gen-Probe Technical Service.

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

**Limitations**

A. This method has been tested using endocervical and male urethral swab specimens only. Performance with other specimens, including conjunctival 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 mix-up, 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 PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

B. The PCA assay differs from other competition assays in that it employs the same nucleic acid detection systems as the primary assay (i.e., PACE 2).

<sup>1</sup> **Note:** A small fraction of samples may yield results between 200 and 350 RLU in the PACE 2 CT assay. 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). For such samples, the value of Tube A must be ≥ 200 RLU in order to evaluate the percent competition value. 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).

- 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. The PCA 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.
- E. 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.
- F. 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.
- G. 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. Clinical Results

The GEN-PROBE PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay was compared to standard culture methods and to the PACE 2 assay for *C. trachomatis* using a total of 133 endocervical and male urethral swab specimens testing positive by cell culture and the PACE 2 assay.

The specimens were categorized as being positive ( $\geq 350$  RLU over the Negative Reference in Tube A and competition  $\geq 70\%$  between Tube A and Tube B), or negative ( $\geq 350$  RLU over the Negative Reference in Tube A, but competition  $< 70\%$  between Tube B and Tube A). Comparisons of the PCA results from each site to standard culture methods and to the PACE 2 assay for *C. trachomatis* are shown below.

### PACE 2 Positive and Culture Positive

Site	Number of Specimens Tested	Number Positive in PCA	Number Negative in PCA
A	21	21	0
B	9	9	0
C	23	23	0
D*	28	28	0
E	39	37	2**
F	13	13	0
<b>TOTAL</b>	<b>133</b>	<b>131</b>	<b>2</b>

\*Site D compared the PCA assay to PACE 2 and Chlamydiazyme (Abbott Laboratories) positive samples.

\*\*One specimen did not repeat positive in Tube A (i.e., below 350 net RLU). The second specimen was positive in Tube A, but showed competition below the 70% cut-off. Data could not be reconciled because sample volumes were insufficient for repeat testing.

### Summary of Competition Values of Positive Specimens

Site	Number of Specimens	Percent Competition	
		Average	Range
A	21	96.6%	78.8 - 98.7%
B	9	97.8%	95.3 - 98.7%
C	23	97.0%	92.4 - 98.5%
D*	28	96.5%	83.5 - 98.7%
E	37	98.0%	91.7 - 105.6%
F	13	97.4%	91.3 - 99.3%
<b>TOTAL</b>	<b>131</b>	<b>97.2%</b>	<b>78.8 - 105.6%</b>

\*Site D compared the PCA assay to PACE 2 and Chlamydiazyme (Abbott Laboratories) positive samples.

### B. DETECTION OF FALSE POSITIVE SPECIMENS

A separate clinical study was conducted to test the ability of PCA to identify PACE 2 false positive samples relative to cell culture. A total of 606 endocervical swab specimens were collected. Samples were first tested in the PACE 2 assay for *C. trachomatis*. Specimens yielding positive PACE 2 results were submitted for cell culture and tested in the PCA assay.

Complete cell culture, PACE 2 and PCA results were obtained for 59 specimens. Of these, four were identified as false positives (culture negative, PACE 2 positive, PCA "A"  $\geq 350$  RLU over the Negative Reference in Tube A, but  $< 70\%$  competition). The PCA "A" tube values for the four false positives ranged from 669 RLU to 795 RLU over the mean of the Negative Reference. The percent competition values for the four samples ranged from 24.2% to 54.9%.

## Analytical Performance Characteristics

### A. Within-Run Precision

The within-run precision of the GEN-PROBE PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay was calculated by assaying a low concentration of *Chlamydia trachomatis* rRNA using ten replicates in a single assay.

Number of Replicates	10
Mean Response (% Competition)	99.9%
Standard Deviation	1.5
Coefficient of Variation	1.5%

### B. Between-Run Precision

Between-run precision was calculated by assaying the same concentration of *C. trachomatis* rRNA on 3 days by three different operators.

Day/Operator	1	2	3
Number of Replicates	10	10	10
Mean Response (% Competition)	99.9%	94.3%	101.1%
Standard Deviation	1.5	3.5	3.8
Coefficient of Variation	1.5%	3.7%	3.8%

### C. Analytical Sensitivity

The analytical sensitivity (limits of detection) of the GEN-PROBE PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay was determined by directly comparing dilutions of freshly grown *C. trachomatis* in cell culture and in the PCA 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

Based on the results of specificity testing of the GEN-PROBE PACE 2 System for CHLAMYDIA TRACHOMATIS, a limited number of closely related and urogenital organisms were selected for testing in the probe competition assay. Testing of *Neisseria gonorrhoeae*, *Chlamydia psittaci*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, and *Candida albicans* in the GEN-PROBE PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay yielded no cross-reactions.

#### E. Recovery

Ribosomal RNA isolated from *Chlamydia psittaci*, *Ureaplasma urealyticum*, and *Neisseria gonorrhoeae* was added at either 0.1 µg/assay or 1.0 µg/assay to samples containing different concentrations of *C. trachomatis* rRNA. As well, *Gardnerella vaginalis* was tested at a concentration of 1 million cells per assay. These additions did not interfere with the recovery of *C. trachomatis* rRNA using the GEN-PROBE PACE 2 CHLAMYDIA TRACHOMATIS Probe Competition Assay.

## Bibliography

- Beebe, J. L., T. R. Sharpton, S. N. Zanto, R. S. Steece, C. Rogers, and S. L. Mottice.** 1997. Performance characteristics of the Gen-Probe competition assay used as a supplementary test for the Gen-Probe PACE 2 and 2C assays for detection of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **35**:477-478.
- Beem, M. O., and E. M. Saxon.** 1977. Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *NEJM* **296**:306-310.
- Cates, Jr., W., and J. N. Wasserheit.** 1991. Genital chlamydia infections: epidemiology and reproductive sequelae. *Am. J. Obstet. Gynecol.* **164**:1771-1781.
- Centers for Disease Control and Prevention.** 1988. United States Morbid. and Mortal. Weekly Rep. **37**:377-382, 387-388.
- Centers for Disease Control and Prevention.** 1993. United States Morbid. and Mortal. Weekly Rep. **42**:(RR-12):1-39.
- Centers for Disease Control and Prevention.** 2007. *Sexually Transmitted Disease Surveillance 2006*. Atlanta, GA: U.S. Department of Health and Human Services. November.
- Frommell, G. T., R. Rothenberg, S. Wang, and K. McIntosh.** 1979. Chlamydial infection of mothers and their infants. *Journal of Pediatrics* **95**:28-32.
- Holmes, K. K.** 1981. The Chlamydia epidemic. *J. Am. Med. Assoc.* **245**:1718-1723.
- Holmes, K. K., H. H. Handsfield, S.P. Wang, B. B. Wentworth, M. Turck, J. B. Anderson, and E. R. Alexander.** 1975. Etiology of nongonococcal urethritis, *NEJM* **292**:1199-1205.
- Kluytmans, J.A.J.W., W.H.F. Goessens, J.H. Van Rijsoort-Vos, H.G.M. Niesters, and E. Stolz.** 1994. Improved performance of PACE 2 with modified collection system in combination with probe competition assay for detection of *Chlamydia trachomatis* in urethral specimens from males. *J. Clin. Microbiol.* **32**:568-570.
- Kohne, D. E., A. G. Steigerwalt, and D. J. Brenner.** 1984. Nucleic acid probe specific for members of the genus *Legionella*: p. 107-108. *In* C. C. Thornsberry, et al. (ed.), *Legionella*: proceedings of the second international symposium. American Society for Microbiology, Washington, D. C.
- Schachter, J.** 1978. Medical progress: chlamydial infections (second of three parts). *NEJM* **298**:490-495.
- Schachter, J.** 1978. Medical progress: chlamydial infections (third of three parts). *NEJM* **298**:540-549.
- Schachter, J., E. C. Hill, E. B. King, V. R. Coleman, P. Jones, and K.F. Meyer.** 1975. Chlamydial infection in women with cervical dysplasia. *Am. J. Obstet. Gynecol.* **123**: 753-757.
- Schachter, J., and M. Grossman.** 1981. Chlamydial infections. *Ann. Rev. Med.* **32**:45-61.

Developed, Manufactured,  
and Distributed by:



Gen-Probe Incorporated  
San Diego, CA 92121 USA



U.S. and international contact information:

Customer Service:	+1 858 410 8002
	customerservice@gen-probe.com
Technical Support:	+1 858 410 8511
	technicalsupport@gen-probe.com

Toll-free from the U.S and Canada:

Customer Service:	+1 800 523 5001
Technical Support:	+1 888 484 4747

[www.gen-probe.com](http://www.gen-probe.com)

This product and its use are covered under one or more of the following patents: U.S. Patent No. 5,599,667, 5,693,468, 5,714,324, 5,723,597, 5,840,488, 6,150,517, 7,087,742, 7,090,972, and 7,138,516; and foreign counterparts.

©1994-2009 Gen-Probe Incorporated

501684EN Rev. A

2009-02