



PACE® 2C CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE

For *in vitro* diagnostic use.

Intended Use

The GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE is a DNA probe test that utilizes nucleic acid hybridization technology to screen for the presence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* from endocervical and male urethral swab specimens collected with the GEN-PROBE PACE Specimen Collection Kits. Follow-up testing in individual *C. trachomatis* and *N. gonorrhoeae* assays is needed to identify the organism(s) present in PACE 2C-positive specimens.

Summary and Explanation of the Test

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are two of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 1,030,911 new cases of *C. trachomatis* and 358,366 new cases of *N. gonorrhoeae* infections were reported in 2006 (8).

The *C. 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 (26). Other clinical symptoms produced by *C. trachomatis* in humans include epididymitis, proctitis, cervicitis, acute salpingitis, and pelvic inflammatory disease (3, 12, 24, 25, 27). In addition to the sexual transmission of chlamydial infections, newborn children are significantly at risk for inclusion conjunctivitis and chlamydial pneumonia from infected mothers (1, 10, 11, 28).

Historically, several methods for *C. trachomatis* detection have been utilized in the clinical laboratory, including cell culture, direct fluorescent antibody testing, and enzyme immunoassay. More recent methodologies for *C. trachomatis* 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 *C. trachomatis*. Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe and NAATs.

Neisseria gonorrhoeae is the causative agent of gonorrhea. This sexually transmitted disease usually results in anterior urethritis accompanied by a purulent exudate in men. In women, the disease is most often found in the cervix, but the vagina and uterus also may be infected. While severe complications and sterility can occur in untreated individuals, asymptomatic infections are frequently diagnosed. Gonorrhea infections also may be diagnosed from other mucous membranes including the conjunctiva, anus, and oropharynx (18).

N. gonorrhoeae is a Gram-negative, oxidase-positive diplococcus that has stringent growth requirements (4, 14, 17, 29, 31). Presumptive diagnosis of gonorrhea is based on recovery of the organism from culture, morphological examination using gram stain, and determination of the presence of cytochrome oxidase (4, 14, 19). Additionally, other confirmatory procedures for the definitive diagnosis of gonorrhea infections include fluorescent antibody staining, carbohydrate degradation, agglutination, sugar fermentation, and nucleic acid hybridization tests (9, 13, 16, 20, 23, 30). More recently, nucleic acid

hybridization has been used to diagnose gonorrhea infections directly from patient samples (22).

The GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE uses nucleic acid hybridization technology (15) to detect *C. trachomatis* and/or *N. gonorrhoeae* directly from endocervical and male urethral swab specimens. The assay does not distinguish between the two organisms, but indicates if one or both are present in a specimen.

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 (15). The GEN-PROBE PACE 2C System uses single-stranded DNA probes with chemiluminescent labels that are complementary to the ribosomal RNA of the target organisms. After the ribosomal RNA is released from the organisms, the labeled DNA probes combine with the ribosomal RNA of the target organisms to form stable DNA:RNA hybrids. The labeled DNA:RNA hybrids are separated from the non-hybridized probes and are measured in the 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 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE Kit

100 test kit (Cat. No. 103905; bioMérieux ref. 39210)

1000 test kit (U.S. and Canada; Cat. No. 103905B)

Store at 2°C to 8°C upon receipt

Symbol	Component	Quantity		Description
		100 tests	1000 tests	
P	PACE 2C Chlamydia trachomatis / Neisseria gonorrhoeae Probe Reagent	2 x 6 mL when reconstituted	20 x 6 mL when reconstituted	Lyophilized, labeled, non-infectious DNA probe (< 500 ng/vial).
HB	PACE 2 Hybridization Buffer	2 x 6 mL	20 x 6 mL	Buffered solution containing < 20% detergent.
S	PACE 2 Selection Reagent	1 x 100 mL	10 x 100 mL	Buffered solution containing < 8% detergent.
SR	PACE 2 STD Separation Reagent	1 x 9 mL	10 x 9 mL	Solid phase (1.25 mg/mL) in a solution containing 0.02% sodium azide as a preservative.

Symbol	Component	Quantity		Description
		100 tests	1000 tests	
W	PACE 2 STD Wash Solution	3 x 200 mL	2 x 3800 mL	Buffered solution containing < 2% detergent.
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 containing < 5% detergent.
PGC	PACE 2 Neisseria gonorrhoeae Positive Control	1 x 3 mL	10 x 3 mL	Non-infectious <i>N. gonorrhoeae</i> nucleic acid in a buffered solution containing < 5% detergent.
NR	PACE 2 STD Negative Reference	1 x 7 mL	10 x 7 mL	Non-infectious nucleic acid in a buffered solution containing < 5% detergent.
	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)

GEN-PROBE PACE 2 CHLAMYDIA TRACHOMATIS kit (Cat. No. 201792/bioMérieux ref. 39211, 201792B)

GEN-PROBE PACE 2 NEISSERIA GONORRHOEAE kit (Cat. No. 201793/bioMérieux ref. 39212, 201793B)

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; 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)

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 and male urethral swab specimens only. Performance with other specimen types has not been assessed.
- 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. Use universal precautions (5). 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 contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping 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

PACE 2C *Chlamydia trachomatis*/*Neisseria gonorrhoeae* Probe Reagent and PACE 2 Separation Reagent must be stored at 2°C to 8°C.

The PACE 2C *Chlamydia trachomatis*/*Neisseria gonorrhoeae* 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 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE 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 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE is designed to screen for the presence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* in endocervical and male urethral specimens collected using the GEN-PROBE PACE Specimen Collection Kits.

Only swabs contained in the PACE Specimen Collection Kits 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

- 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.
- Insert the second swab from the collection kit into the endocervical canal.
- Rotate the swab for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
- Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
- Fully insert** the swab into the GEN-PROBE transport tube.
- 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

- Patient should not have urinated for at least 1 hour prior to sample collection.
- Insert the swab from the urethral/conjunctival collection kit 2 to 4 cm into the urethra using a rotating motion to facilitate insertion.
- 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.
- Withdraw the swab.
- Fully insert** the swab into the GEN-PROBE transport tube.
- 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 2C 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 medium) may interfere with performance.

D. Specimens must be shipped in accordance with applicable national and international transportation regulations. Store and test as described (see B above).

Test Procedure

A. Sample Preparation

- Allow the specimens to reach room temperature prior to processing.
- Vortex each GEN-PROBE transport tube for at least 5 seconds.
- Express all liquid from the swab by pressing the swab against the wall of the tube. Discard the swab.
- Prior to testing, vortex the transport tube for at least 5 seconds to ensure homogeneity.

B. Reagent Preparation

- 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 PACE 2C *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Probe Reagent (PACE 2C 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 PACE 2C 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 PACE 2C 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 PACE 2C 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 = 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

- Label tubes with sample identification numbers. Include three tubes for the Negative Reference, one for the *C. trachomatis* Positive Control, and one for the *N. gonorrhoeae* Positive Control. Label near the tops of the tubes only. The reference and controls must be run with each batch of specimens.
- Insert the tubes into the tube rack portion of the GEN-PROBE Magnetic Separation Unit. Set aside the base portion of the separation unit for later use.
- Vortex each specimen for 5 seconds.
- Pipette 100 μ L of each of the controls and specimens to the bottom of the respective tubes.
- Pipette 100 μ L of the PACE 2C Probe Reagent to the BOTTOM of each tube, taking care not to touch the top or sides of the tube.
- Cover the tubes with Sealing Cards ensuring that each tube is sealed.
- Shake the rack 3 to 5 times to mix.
- Incubate the tubes in a water bath at $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 1 hour. Do **NOT** place the magnetic separation unit base in the water bath.

D. Equipment Preparation

- Prepare the GEN-PROBE LEADER luminometer for operation. Make sure there is sufficient volume of Detection Reagents I and II to complete the tests.

E. Separation

- Remove the tube rack from the water bath and remove the Sealing Cards.
- Pipette 1 mL of the well-mixed, prepared Separation Suspension into each tube.
- 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.
- Immediately incubate the tubes in a water bath at $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 10 minutes.
- 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.
- 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.
- 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.
- Allow the tubes to remain on the magnetic separation base for 20 minutes at room temperature.

- 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 μ L of Wash Solution should remain in each tube.
- Separate the tube rack from the base and shake the tube rack to resuspend the pellets.

F. Detection

- Select the appropriate protocol from the LEADER luminometer software.
- 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.
- Ensure that the pellets are resuspended and insert the tubes in the LEADER luminometer according to the prompts provided by the instrument software.
- Read the tubes in the following order:
 - Negative Reference, 3 tubes
 - C. trachomatis* Positive Control, 1 tube
 - N. gonorrhoeae* Positive Control, 1 tube
 - Specimen tubes
- When the analysis is complete, remove the tube(s) from the LEADER luminometer.

Procedural Notes

A. PACE 2 Hybridization Buffer and PACE 2C Probe Reagent

Gel formation of the PACE 2 Hybridization Buffer and reconstituted PACE 2C 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 PACE 2C Probe Reagent, Separation Suspension, and Wash Solution. Pipettors with disposable tips are recommended for pipetting specimens, references, and controls to avoid sample carry-over and cross-contamination. Care should be taken to pipette PACE 2C 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

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 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE 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:

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

$$\text{Assigned Cut-off} = 300 \text{ RLU}$$

$$\text{Calculated Cut-off} = 300 \text{ RLU} + 72 \text{ RLU} = 372 \text{ RLU}$$

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

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

B. Interpretation of Results

Results from the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

POSITIVE — The difference is ≥ 300 RLU.

NEGATIVE — The difference is < 300 RLU.

A positive PACE 2C screening result may indicate the presence of *Chlamydia trachomatis* rRNA and/or *Neisseria gonorrhoeae* rRNA in the specimen tested. Follow-up testing in individual *C. trachomatis* and *N. gonorrhoeae* assays is needed to identify the organism(s) (7).

Results of the follow-up identification tests should be used to determine if the specimens contain *C. trachomatis* and/or *N. gonorrhoeae*. Follow the “Results Interpretation” instructions in the follow-up test package inserts.

A negative result should be reported that *Chlamydia trachomatis* rRNA and *Neisseria gonorrhoeae* rRNA were not detected in the specimen tested.

C. Quality Control and Acceptability of Results

Note: The Negative Reference and Positive Control provided, control the PACE 2C assay only. They do not control for the lysis of the target organism(s) in the specimen transport medium.

Negative Reference

The Negative Reference provides a measure of the assay background and is used to calculate the run cut-off. The expected values of the Negative Reference were validated using 69 runs (3 replicates/run) at five locations throughout the United States.

The response of each Negative Reference 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 Variance 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 contact GEN-PROBE Technical Support if the problem persists.

Positive Controls

The expected values for each of the Positive Controls were validated using 69 different runs at five locations throughout the United States.

The difference in the response of each of the Positive Controls and the mean response of the Negative Reference should be > 600 RLU and < 3,200 RLU or the run is invalid. If the Positive Control values repeatedly fall out of specification, contact Gen-Probe Technical Support. Results from the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

If the Positive Controls or Negative Reference values are not in the required ranges, the test results are invalid and must not be reported.

Each laboratory under its normal operating conditions should establish its own mean and range for the Negative Reference and Positive Controls and maintain records according to Standard Laboratory Quality Control practices (2, 21).

Sample Processing Cell Controls

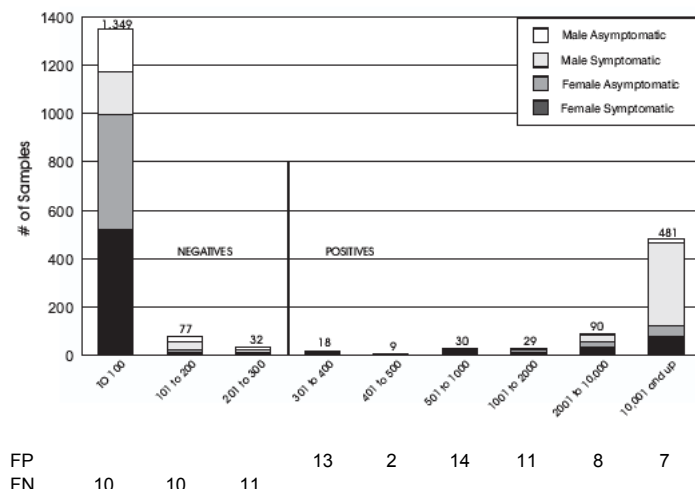
To test the effectiveness of sample processing, positive and negative cell controls may be run in conjunction with the specimens. For example, *C. trachomatis* ATCC VR878 and *N. gonorrhoeae* ATCC 19424 may be used as the positive cell controls and *N. mucosa* ATCC 19696 may be used as the negative cell control. For the Neisseria isolates, use actively growing cultures to prepare suspensions of approximately 3 x 10⁸ CFU/mL in sterile saline. Inoculate 10 µL of each cell suspension into a transport tube from a GEN-PROBE PACE Specimen Collection Kit (endocervical or urethral/conjunctival) and vortex. For the Chlamydia isolates, use stocks harvested from cell culture and titered to be approximately 3 x 10⁸ IFU/mL. Transfer 10 µL of each stock to a transport tube from a GEN-PROBE PACE Specimen Collection Kit (endocervical or urethral/conjunctival) and vortex. Process the controls in the same manner as the patient specimens, beginning with Step C1 of the test procedure. The positive cell controls should produce positive test results and the negative cell controls should produce negative test results.

- F. As in any disease state, the positive predictive value of this assay will decrease as the prevalence decreases in the population. 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. See the *Specimen Collection and Preparation* section of this insert for instructions.
- G. Therapeutic failure or success cannot be determined as nucleic acid may persist following appropriate antimicrobial therapy.
- H. Results from the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- I. A negative test does not exclude the possibility that the numbers of *C. trachomatis* and/or *N. gonorrhoeae* organisms may be below the level of detection of the assay. A second swab can be collected and cultured to identify those patients infected with low levels of organism(s). As well, test results may be affected by improper specimen collection, technical error, specimen mix-up or concurrent antibiotic therapy.
- J. If a positive PACE 2C result contradicts other clinical or patient information or if the patient belongs to a category cited in the 1993 CDC *C. trachomatis* guidelines (6), verification of the result may be warranted.
- K. Because the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE is a screening assay designed to detect the presence of *C. trachomatis* and/or *N. gonorrhoeae*, and because of the possibility of dual infection, follow-up testing of PACE 2C-positives in individual *C. trachomatis* and *N. gonorrhoeae* assays is needed.

Clinical Performance Characteristics

A. Distribution of Clinical Results

Using the results obtained for the specimens tested in the clinical trial for PACE 2C, a distribution of sample RLU values above and below the assay cut-off was generated. The data are presented below after resolution of discrepant specimens. The numbers of false positive (FP) and false negative (FN) results for each RLU category are given below the figure.



Limitations

- A. This method has been tested using endocervical and male urethral swab specimens only. Performance with other specimens has not been assessed.
- B. 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.
- C. The PACE 2C 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. Other endogenous substances that may be present in patient samples may interfere with the assay.
- E. All *Chlamydia trachomatis* and *Neisseria gonorrhoeae* 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 and gonorrheal infection in medicolegal cases.

Analytical Performance Characteristics

A. Within-Run Precision

The within-run precision of the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE was calculated by assaying four concentrations of *C. trachomatis* inclusion forming units (IFU) and *N. gonorrhoeae* cells using five replicates in a single assay; one negative sample was also run.

Sample	A	B	C	D	E
Number of Replicates	5	5	5	5	5
Mean Response (RLU)	9530	2075	1041	767	49
Standard Deviation (RLU)	210	143	42	52	2
Coefficient of Variance	2.2%	6.9%	4.0%	6.8%	n/a

B. Between-Run Precision

Between-run precision was calculated by assaying the same four concentrations of *C. trachomatis* IFU and *N. gonorrhoeae* cells and one negative sample using the average of five replicates determined in three consecutive runs.

Sample	A	B	C	D	E
Number of Replicates	3	3	3	3	3
Mean Response (RLU)	10,147	2259	968	690	51
Standard Deviation (RLU)	917	240	66	74	4
Coefficient of Variance	9.0%	10.6%	6.8%	10.7%	n/a

C. Positive Control Precision

Precision data for the *C. trachomatis* and *N. gonorrhoeae* Positive Controls were determined in PACE 2C assays performed at five locations throughout the United States. One replicate of each Positive Control was assayed in each PACE 2C run.

Sample	<i>C. trachomatis</i> Positive Control	<i>N. gonorrhoeae</i> Positive Control
Number of Replicates	69	69
Mean Response (RLU)	1837	2165
Standard Deviation (RLU)	329	425
Coefficient of Variance	5.6%	5.1%

D. Analytical Sensitivity

The analytical sensitivity (limits of detection) of the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE was determined by directly comparing dilutions of freshly grown *C. trachomatis* and *N. gonorrhoeae* in cell culture and in the PACE 2C assay. The sensitivities for the 15 *C. trachomatis* serovars at the assay cut-off of 300 RLU plus the mean of the Negative Reference ranged from 24–2,232 inclusion-forming units (IFU)/assay (0.1 mL inoculated transport medium); the average was 966 IFU/assay. The sensitivity of *N. gonorrhoeae* was determined to be approximately 650 colony-forming units (CFU)/assay (0.1 mL inoculated transport medium).

E. Analytical Specificity

A total of 80 culture isolates were evaluated using the PACE 2C assay. These isolates included 20 organisms that may be isolated from the urogenital tract and 30 additional organisms that represent a phylogenetic cross-section of organisms. Culture isolates of *C. trachomatis* (15 serovars), *N. gonorrhoeae*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, and 12 species of *Neisseriaceae* were also tested. Only the *C. trachomatis* and *N. gonorrhoeae* samples produced a positive result in the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE.

F. Recovery

Ribosomal RNA isolated from *C. psittaci*, *Ureaplasma urealyticum*, and *Neisseria meningitidis* was added at a concentration of 0.1 µg/ assay to samples containing different concentrations of *C. trachomatis* and/or *N. gonorrhoeae* ribosomal RNA. These additions did not interfere with the recovery of *C. trachomatis* or *N. gonorrhoeae* rRNA using the PACE 2C assay.

G. Clinical Trial Results

The GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE was compared to standard culture methods for *C. trachomatis* and *N. gonorrhoeae* using 1,266 endocervical specimens and 849 male urethral specimens. Specimens were evaluated at a total of five clinical sites using a 300 net RLU cut-off. Clinical data are presented below, both before and after resolution of discrepant specimens.

1. Performance Summary: Before Discrepant Resolution

PACE 2C Culture	Pos Pos	Pos Neg	Neg Pos	Neg Neg	Sensitivity / Specificity (%)
Population (%CT / %NG prevalence; # sites)					
Female Symptomatic					
High Prevalence (13.6% / 16.2%; 2 sites)	74	9	6	219	92.5 / 96.1
Low Prevalence (8.9% / 7.0%; 2 sites)	48	8	3	313	94.1 / 97.5
Female Asymptomatic					
High Prevalence (12.1% / 17.0%; 2 sites)	71	6	8	221	89.9 / 97.4
Low Prevalence (3.2% / 1.8%; 2 sites)	12	4	1	263	92.3 / 98.5
Male Symptomatic (11.5% / 55.7%; 4 sites)					
	371	17	11	208	97.1 / 92.4
Male Asymptomatic (6.6% / 5.4%; 4 sites)					
	26	11	2	203	92.9 / 94.9
Combined (10.1% / 41.3%; 4 sites)					
	397	28	13	411	96.8 / 93.6

2. Performance Summary: After Discrepant Resolution

Discrepant samples for *C. trachomatis* were resolved by re-culture and DFA. Cell culture was not repeated for *N. gonorrhoeae* discrepant samples.

PACE 2C Culture	Pos	Pos	Neg	Neg	Sensitivity /Specificity (%)	95% Confidence Intervals Sensitivity / Specificity (%)
	Pos	Neg	Pos	Neg		
Population						
Female Symptomatic						
High Prevalence	77	6	6	219	92.8/97.3	86.7–97.9 / 94.7–99.1
Low Prevalence	49	7	3	313	94.2/97.8	86.5–100.0 / 95.6–99.1
Female Asymptomatic						
High Prevalence	71	6	8	221	89.9/97.4	81.0–94.9 / 94.7–99.1
Low Prevalence	13	3	1	263	92.9/98.9	71.4–100.0 / 97.4–100.0
Male Symptomatic						
	373	15	11	208	97.1/93.3	95.3–98.7 / 89.7–96.0
Male Asymptomatic						
	28	9	2	203	93.3/95.8	83.3–100.0 / 92.5–98.1
Combined						
	401	24	13	411	96.9/94.5	94.9–98.3 / 92.0–96.3

Of the 55 total PACE 2C-positive, culture-negative samples, 15 were negative when tested in the individual PACE 2 assays for *C. trachomatis* and *N. gonorrhoeae*. As well, although it could not be included in the discrepant resolution protocol described above, a research amplification assay was used to test a number of the apparent PACE 2C false positives. Of 40 PACE 2C-positive, culture-negative probe samples tested in amplification, 26 demonstrated the presence of *C. trachomatis* nucleic acid and 11 *N. gonorrhoeae* nucleic acid. These data indicate that a majority of samples classified in the above table as PACE 2C false positives were, in fact, true positives that were missed by cell culture. The samples remained classified as false positives because the amplification assay used was a research assay.

3. Performance Summary: Individual Organisms

The ability of PACE 2C to detect *C. trachomatis* and *N. gonorrhoeae* individually was determined by analyzing the resolved PACE 2C vs. culture results of positive samples separately for each of the two target organisms. In order to simplify the analysis, dual positive samples were included in both the *C. trachomatis* and *N. gonorrhoeae* data. Therefore, the total number of positive samples in the table below is increased by 59 over the number in the other clinical data tables.

	PACE 2C	
	Positive	Negative
<i>C. trachomatis</i> Culture Positive		
Female	109	12
Male	79	12
<i>N. gonorrhoeae</i> Culture Positive		
Female	132	6
Male	350	1

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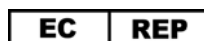
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