



PACE® 2 NEISSERIA GONORRHOEAE

For *in vitro* diagnostic use.

Intended Use

The GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE is a rapid DNA probe test that utilizes the technique of nucleic acid hybridization for the detection of *Neisseria gonorrhoeae* in female endocervical and male urethral swab specimens and identification of *Neisseria gonorrhoeae* from culture isolates.

Summary and Explanation of the Test

Neisseria gonorrhoeae infections are one of the most commonly reported sexually transmitted infections worldwide. In the United States alone, an estimated 358,366 new cases of gonorrhea were reported in 2006 (1). 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 (8).

Neisseria gonorrhoeae is the causative agent of gonorrhea. *N. gonorrhoeae* is a Gram-negative, oxidase-positive diplococcus that has stringent growth requirements (3, 5, 7, 12, 14). 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 (3, 5, 9). Additionally, other confirmatory procedures for the definitive diagnosis of gonorrhea infections include fluorescent antibody staining, carbohydrate degradation, agglutination and sugar fermentation tests (2, 4, 10, 11, 13).

The GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE uses the technique of nucleic acid hybridization (6) to identify *Neisseria gonorrhoeae* directly from endocervical and male urethral swab specimens and to identify *Neisseria gonorrhoeae* isolated from culture.

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 (6). 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 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 NEISSERIA GONORRHOEAE Kit

100 test kit (Cat. No. 201793; bioMérieux ref. 39212)

1000 test kit (Cat. No. 201793B)

2°C to 8°C

Symbol	Component	Quantity		Description
		100 tests	1000 tests	
P	PACE 2 Neisseria gonorrhoeae Probe Reagent	2 x 6 mL when reconstituted	20 x 6 mL when reconstituted	Contains labeled, lyophilized <i>N. gonorrhoeae</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.
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.
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 NEISSERIA GONORRHOEAE Probe Competition Assay Kit (Cat. No. 103549)

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 and culture isolates 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 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 2 System for NEISSERIA GONORRHOEAE is designed to detect the presence of *Neisseria gonorrhoeae* in specimens obtained from the male urethra and the female endocervical canal using the GEN-PROBE PACE Specimen Collection Kit. The PACE 2 assay may also be used to identify *N. gonorrhoeae* from culture isolates.

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 score line 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. Collect the urethral exudate or 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 score line to fit the tube; use care to avoid splashing of contents. **Cap the tube tightly.**
- 3. Culture isolates from modified Thayer-Martin or chocolate agar plates
 - a. Using an inoculating loop, remove a sufficient number of colonies from a culture plate that has been properly incubated for no longer than 24 hours (6). Prepare a bacterial suspension equal to a #1 McFarland standard in sterile saline and vortex.
 - b. Pipette 100 µL of the prepared cell suspension into a GEN-PROBE transport tube and vortex.
- 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 843895. Store and test as described above.

Test Procedure

A. Sample Preparation

Allow the specimens to reach room temperature prior to processing.

- 1. Swabs
 - a. Vortex each GEN-PROBE transport tube for at least 5 seconds.
 - b. Express all liquid from the swab by pressing the swab against the wall of the tube. Discard the swab.
 - c. Prior to testing, vortex the transport tube for at least 5 seconds to ensure homogeneity.
- 2. Culture isolates
 - a. Vortex the GEN-PROBE transport tube 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 Magnetic Separation Unit together, decant the supernatants. Before turning the 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 μL 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. Please note that separate protocols are required for specimens and culture isolates.
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 tubes in 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 the Hybridization Buffer and reconstituted Probe Solution 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 reagent 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 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 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 on 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 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:

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

$$\text{Specimen Response} = 894 \text{ RLU}$$

$$\text{Difference} = 894 \text{ RLU} - 55 \text{ RLU} = 839 \text{ RLU} \quad \text{Positive}$$

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

1. Direct Specimen
 - POSITIVE - The difference is ≥ 300 RLU.
 - NEGATIVE - The difference is < 300 RLU.
2. Culture Confirmation
 - POSITIVE - The difference is $\geq 10,000$ RLU.
 - NEGATIVE - The difference is $< 10,000$ RLU.

A positive result indicates that *Neisseria gonorrhoeae* is present in the specimen tested and strongly supports a diagnosis of gonorrheal infection.

A negative result indicates the absence of *Neisseria gonorrhoeae* in the specimen tested.

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 $\leq 30\%$). 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 NEISSERIA GONORRHOEAE Probe Competition Assay (PCA) can be used in conjunction with the PACE 2 System for NEISSERIA GONORRHOEAE as a supplemental test to detect nonspecific signal in endocervical and male urethral swab specimens and in culture isolates. 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.

Limitations

- A. This method has been tested using endocervical and male urethral swab specimens and culture isolates 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, or 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 NEISSERIA GONORRHOEAE 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 μL 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 *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 gonorrheal infection in cases of suspected child abuse.
- E. When using the culture confirmation procedure, the organism must be viable to obtain a valid result.
- 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 gonorrheal 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

The GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE was compared to standard culture methods using urethral specimens obtained from 387 men and 1375 endocervical specimens obtained from women.

The specimens were categorized as either positive (difference ≥ 300 RLU) or negative (difference < 300 RLU). A comparison of these results to standard culture methods is shown below.

A. Performance Summary with Initial Data
Clinical Specimens

Low Positivity Rate (< 10%)

PACE 2 / Culture						
PACE 2 Culture	Pos	Pos	Neg	Neg	Sensitivity / Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
Population						
Women (3%)	24	5	1	872	96.0% / 99.4%	99%

High Positivity Rate (> 10%)

PACE 2 / Culture						
PACE 2 Culture	Pos	Pos	Neg	Neg	Sensitivity / Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
Population						
Men (29%)	110	7	1	269	99.1% / 97.5%	98%
Women (23%)	99	7	10	357	90.8% / 98.1%	96%

Combined High and Low Positivity Rate

PACE 2 / Culture						
PACE 2 Culture	Pos	Pos	Neg	Neg	Sensitivity / Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
Population						
Men (29%)	110	7	1	269	99.1% / 97.5%	98%
Women (10%)	123	12	11	1229	91.8% / 99.0%	98%
Total (14%)	233	19	12	1498	95.1% / 98.7%	98%

The positive and negative predictive values for various positivity rates at a sensitivity and specificity of 95.1% and 98.7% are shown below.

Predictive Values

	POSITIVITY RATE			
	5%	10%	15%	20%
Positive Predictive Value (%)	80.0	89.4	93.1	95.0
Negative Predictive Value (%)	99.7	99.5	99.1	98.8

B. Performance Summary with Discrepant Analysis

Eighteen of the 19 specimens producing an "Apparent False Positive Result" were tested for the presence of gonorrhea specific nucleic acid using other DNA probe techniques. Fifteen of the 18 specimens tested contained *Neisseria gonorrhoeae* specific nucleic acid.

Clinical Specimens

Low Positivity Rate (< 10%)

PACE 2 / Culture						
PACE 2 Culture	Pos	Pos	Neg	Neg	Sensitivity / Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
Population						
Women (3%)	26	3	1	872	96.3% / 99.7%	99%

High Positivity Rate (> 10%)

PACE 2 / Culture						
PACE 2 Culture	Pos	Pos	Neg	Neg	Sensitivity / Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
Population						
Men (30%)	116	1	1	269	99.1% / 99.6%	99%
Women (25%)	106	0	10	357	91.4% / 100.0%	98%

Combined High and Low Positivity Rate

PACE 2 / Culture						
PACE 2 Culture	Pos	Pos	Neg	Neg	Sensitivity / Specificity	Percent Agreement
	Pos	Neg	Pos	Neg		
Population						
Men (30%)	116	1	1	269	99.1% / 99.6%	99%
Women (10%)	132	3	11	1229	92.3% / 99.8%	99%
Total (15%)	248	4	12	1498	95.4% / 99.8%	99%

The positive and negative predictive values for various positivity rates at a sensitivity and specificity of 95.4% and 99.8% are shown below.

Predictive Values

	POSITIVITY RATE			
	5%	10%	15%	20%
Positive Predictive Value (%)	96.2	98.1	98.8	99.2
Negative Predictive Value (%)	99.8	99.5	99.2	98.9

C. Culture Confirmation

A total of 244 culture isolates were evaluated using the GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE and compared to culture. The results are shown below.

PACE 2 Culture	PACE 2 / Culture				Sensitivity / Specificity
	Pos Pos	Pos Neg	Neg Pos	Neg Neg	
	148	1	0	95	100.0% / 99.0%

The one "Apparent False Positive" result was obtained from ATCC 43831 which resembles both *N. gonorrhoeae* and *N. meningitidis*.

Analytical Performance Characteristics

A. Within-Run Precision

The within-run precision of the GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE was calculated by assaying three concentrations of *Neisseria gonorrhoeae* ribosomal RNA using 10 replicates in a single assay.

Sample	A	B	C
Number of Replicates	10	10	10
Mean Response (RLU)	781.1	4403.0	9476.7
Standard Deviation (RLU)	32.1	97.4	186.0
Coefficient of Variation	4.1%	2.2%	2.0%

B. Between-Run Precision

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

Sample	A	B	C
Number of Replicates	12	12	12
Mean Response (RLU)	832.5	4600.1	9635.8
Standard Deviation (RLU)	71.1	384.2	886.9
Coefficient of Variation	8.5%	8.4%	9.2%

C. Analytical Sensitivity

The analytical sensitivity of the GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE was determined by directly comparing dilutions of freshly grown *N. gonorrhoeae* in cell culture and in the PACE 2 assay. The sensitivity at the assay cut-off of 300 Net RLU was 647 colony-forming units (CFU)/assay.

D. Analytical Specificity

A total of 259 culture isolates were evaluated using the GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE. These isolates represented a total of 62 species from 44 genera. Sixteen genera that may be isolated from the urogenital tract and 22 additional genera representing a phylogenetic cross section of organisms were evaluated using the GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE. Sixteen species of Neisseriaceae were tested, including *N. lactamica*, 5 serogroups of *N. meningitidis*, *N. cinerea* and *Kingella* species. Additionally 151 clinical isolates of *N. gonorrhoeae* and 8 ATCC reference strains of *N. gonorrhoeae* were tested. All the cultures of *N. gonorrhoeae* produced a positive result in the GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE. Only one of the other culture isolates produced a positive result. This *Neisseria* isolate is ATCC 43831 that is known to resemble both *N. gonorrhoeae* and *N. meningitidis*.

E. Recovery

Escherichia coli, *Gardnerella vaginalis*, and *Candida albicans* were added at a concentration of 10 million cells per test to samples containing various concentrations of *N. gonorrhoeae* rRNA. These additions did not interfere with the recovery of *N. gonorrhoeae* rRNA using the GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE.

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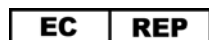
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