



## PACE<sup>®</sup> 2 NEISSERIA GONORRHOEAE Probe Competition Assay

For *in vitro* diagnostic use.

### Intended Use

The PACE 2 NEISSERIA GONORRHOEAE 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 specimens and male urethral specimens that test positive in the PACE 2 System for NEISSERIA GONORRHOEAE. PCA also can be used with the PACE 2 assay for the identification of *Neisseria gonorrhoeae* from culture isolates.

### Summary and Explanation of the Test

Gonorrhea is one of the most commonly reported bacterial infections in the United States, with 358,366 new cases reported in 2006 (2). 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. Gonorrheal infections may be diagnosed from other mucous membranes including the conjunctiva, anus, and oropharynx (9).

*Neisseria gonorrhoeae* is a Gram-negative, oxidase-positive diplococcus that has stringent growth requirements (4, 6, 8, 14, 16). Several methods have been used to detect *N. gonorrhoeae* in patient specimens. Traditionally, presumptive diagnosis is based on recovery of the organism from culture, morphological examination using Gram stain and determination of the presence of cytochrome oxidase (4, 6, 10). Confirmatory procedures for definitive diagnosis of gonorrhea infections include fluorescent antibody staining, carbohydrate degradation, agglutination, and sugar fermentation tests (3, 5, 11, 13, 15). More recently, nucleic acid hybridization has been used to diagnose gonorrhea infections (12). The GEN-PROBE PACE 2 System for NEISSERIA GONORRHOEAE is a nucleic acid hybridization method that uses single-stranded DNA probe with a chemiluminescent label that is complementary to the ribosomal RNA of the target organism (7). 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.

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. PCA can also be used with the PACE 2 assay for the identification of *Neisseria gonorrhoeae* from culture isolates. Samples are first tested in the PACE 2 assay to differentiate positive from negative samples. Positive samples 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 sample contains *N. gonorrhoeae* and is not giving a positive reaction because of nonspecific signal.

### Reagents

Reagents for the PACE 2 NEISSERIA GONORRHOEAE Probe Competition Assay are provided in three separate reagent kits. Also see *Materials Required but not Provided*.

### Materials Provided

**PACE<sup>®</sup> 2 NEISSERIA GONORRHOEAE Probe Competition Assay Kit (Cat. No. 103549)**

2°C to 8°C

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

### Materials

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

### Materials Required but not Provided

- GEN-PROBE<sup>®</sup> PACE<sup>®</sup> 2 System for NEISSERIA GONORRHOEAE (Cat. No. 201793)
- GEN-PROBE<sup>®</sup> PACE<sup>®</sup> Specimen Collection Kits for Male Urethral or Conjunctival Specimens (Cat. No. 103275) (50/box)
- GEN-PROBE<sup>®</sup> PACE<sup>®</sup> Specimen Collection Kits for Endocervical Specimens (Cat. No. 103300) (50/box)
- GEN-PROBE<sup>®</sup> Detection Reagent Kit (Cat. No. 201791) (1200 tests)
- GEN-PROBE<sup>®</sup> LEADER<sup>®</sup> Luminometer
- GEN-PROBE<sup>®</sup> Magnetic Separation Unit (Cat. No. 101639 or equivalent)
- Vortex mixer
- Covered water bath (60°C ± 1°C)
- Micropipettes (100 µL)
- Pipettes capable of delivering 1 to 25 mL
- Lint-free wipes
- PACE 2 Reaction Tubes (Cat. No. 102065)

## Optional Materials

- GEN-PROBE® FAST Express Reagent Kit (Cat. No. 102930)
- GEN-PROBE® STD Proficiency Panel (Cat. No. 102325)
- 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 (1).
- C. This test system has been evaluated using endocervical and male urethral swab specimens and culture isolates 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 these reagents, always dilute the material with a large volume of water to prevent azide buildup in the plumbing.
- F. Avoid contact of Detection Reagents I and II with skin and mucous membranes. Wash with water if these reagents come into contact with skin. If spills of these reagents occur, dilute with water before wiping dry.
- G. 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 in the foil pouches 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 NEISSERIA GONORRHOEAE Probe Competition Assay is designed to detect nonspecific signal in culture isolates and in specimens obtained from the male urethra and the female endocervical canal using the GEN-PROBE PACE Specimen Collection Kit. Samples tested in PCA are those that have been processed and tested in the PACE 2 System for NEISSERIA GONORRHOEAE 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 NEISSERIA GONORRHOEAE package insert may be tested in the PACE 2 NEISSERIA GONORRHOEAE Probe Competition Assay. All specimens must be collected using a GEN-PROBE PACE Specimen Collection Kit.
- B. The PCA assay can be used to test PACE 2 specimens prepared for identification of *Neisseria gonorrhoeae* culture isolates. Specimens should be prepared as directed in the PACE 2 System for NEISSERIA GONORRHOEAE package insert.
- C. 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.

- D. 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.
- E. 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. 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/Culture Isolates/Controls

For each specimen/culture isolate 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 *Neisseria gonorrhoeae* (NG) 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 NG 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 NEISSERIA GONORRHOEAE 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 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 N. GONORRHOEAE pouch. Label the standard PACE 2 reaction tube "PCA Positive A" and label the capped PCA tube from the pouch "PCA Positive B."
  - c. **Samples:** One standard PACE 2 reaction tube and one capped PCA tube from the PCA N. GONORRHOEAE pouch for each sample to be tested. Label each set of samples with the sample 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 µL of PACE 2 Negative Reference according to the directions in the

PACE 2 System for NEISSERIA GONORRHOEAE package insert.

5. To the two PCA Positive Control tubes labeled "A" and "B," add 100  $\mu$ L of PACE 2 *N. gonorrhoeae* Positive Control according to the directions in the PACE 2 System for NEISSERIA GONORRHOEAE 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 NEISSERIA GONORRHOEAE 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 the 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 NEISSERIA GONORRHOEAE package insert.

#### E. 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 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.

## 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.

#### C. Pipetting

For convenience, repeating pipettors or dispensers may be used for addition of the 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 bottom of the tubes 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 the 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

1. Direct Specimens

The results of the GEN-PROBE PACE 2 NEISSERIA GONORRHOEAE Probe Competition Assay are calculated based on the percent difference in 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 300 RLU or greater over the mean negative reference is required in Tube A. Samples yielding less than 300 RLU over the mean negative reference are negative for *N. gonorrhoeae* 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:

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

$$\begin{aligned} \text{Specimen Response Tube A} &= 894 \text{ RLU} \\ \text{Net Signal} &= 894 - 55 = 839 \text{ RLU} \\ \text{Specimen Response Tube B} &= 60 \text{ RLU} \end{aligned}$$

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

The LEADER luminometer prints the sample responses for Tubes A and B. For Tube A, the LEADER luminometer compares the value to an assigned cut-off and prints a positive or negative interpretation. For Tube B, the LEADER luminometer 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.

2. Culture Confirmation

The calculation of results for culture confirmation samples is the same as described for direct specimens except that a value of 10,000 RLU or greater over the mean negative reference is required in Tube A. Samples yielding less than 10,000 RLU over the mean negative reference are negative for *Neisseria gonorrhoeae* and, therefore, the percent competition should not be evaluated.

B. Interpretation of Results

1. Direct Specimen

True = Tube A ≥ 300 net RLU and % Competition ≥ 70%

False = Tube A ≥ 300 net RLU and % Competition < 70%

A true result indicates that the signal obtained using the PACE 2 System for NEISSERIA GONORRHOEAE is the result of the presence of *N. gonorrhoeae* in the sample.

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

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.

2. Culture Confirmation

True = Tube A Signal ≥ 10,000 net RLU and % Competition ≥ 70%

False = Tube A Signal ≥ 10,000 net RLU and % Competition < 70%

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 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 greater than 600 RLU for Tube A. The percent competition must be greater than or equal to 90%. If the Positive Control values repeatedly fall 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.

**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 GEN-PROBE PACE 2 NEISSERIA GONORRHOEAE 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).
- 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 *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.
- 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 NEISSERIA GONORRHOEAE Probe Competition Assay was compared to standard culture methods and to the PACE 2 assay for *N. gonorrhoeae* using a total of 241 endocervical and male urethral swab specimens testing positive by cell culture and the PACE 2 assay.

The specimens were categorized as being positive (≥ 300 RLU over the Negative Reference in Tube A and competition ≥ 70% between Tube A and Tube B) or negative (≥ 300 RLU over the Negative Reference 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 *N. gonorrhoeae* are shown below.

#### PACE 2 Positive and Culture Positive

Site	Number of Specimens Tested	Number of Positive in PCA	Number of Negative in PCA
A	12	12	0
B	106	105	1*
C	14	13	1*
D	109	109	0
<b>TOTAL</b>	<b>241</b>	<b>239</b>	<b>2</b>

\*Data could not be reconciled because sample volumes were insufficient for repeat testing. Although the PCA results of Tube A were below the cut-off, both samples showed competition values of greater than 70%, suggesting the presence of target.

#### Summary of Competition Values of Positive Specimens

Site	Number of Specimens	% Competition	
		Average	Range
A	12	98.4%	97.9 – 99.2%
B	105	98.4%	87.3 – 99.0%
C	13	97.2%	95.7 – 98.4%
D	109	97.9%	94.2 – 99.6%
<b>TOTAL</b>	<b>239</b>	<b>98.1%</b>	<b>95.7 – 99.6%</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 426 endocervical swab specimens were tested in cell culture and in the PACE 2 assay for *N. gonorrhoeae*. Samples testing positive in PACE 2 were then tested in the PCA assay.

Of the 426 samples tested, 3 were identified as false positives (culture negative, PACE 2 positive, PCA "A" ≥ 300 RLU over the Negative Reference in Tube A, but < 70% competition). The PCA "A" tube values for the 3 false positives ranged from 476 RLU to 693 RLU over the mean of the Negative Reference. The percent competition values for the three samples ranged from -3.3% to 21.4%.

### Analytical Performance Characteristics

#### A. Within-Run Precision

The within-run precision of the GEN-PROBE PACE 2 NEISSERIA GONORRHOEAE Probe Competition Assay was calculated by assaying a low concentration of *Neisseria gonorrhoeae* rRNA using 10 replicates in a single assay.

Number of Replicates	10
Mean Response (% competition)	95%
Standard Deviation	3.8
Coefficient of Variation	4.0%

#### B. Between-Run Precision

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

Day/Operator	1	2	3
Number of Replicates	10	10	10
Mean Response (% competition)	95.0%	96.3%	92.1%
Standard Deviation	3.8	0.71	9.0
Coefficient of Variation	4.0%	0.7%	9.8%

#### C. Analytical Sensitivity

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

#### D. Analytical Specificity

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

#### E. Recovery

Ribosomal RNA isolated from *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Neisseria meningitidis* was added at either 0.1 µg/assay or 1.0 µg/assay to samples containing different concentrations of *N. gonorrhoeae* 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 *N.*

*gonorrhoeae* rRNA using the GEN-PROBE PACE 2 NEISSERIA GONORRHOEAE Probe Competition Assay.

## Bibliography

1. **Centers for Disease Control and Prevention.** 1988. United States Morbid. and Mortal. Weekly Rep. **37**:377-382, 387-388.
2. **Centers for Disease Control and Prevention.** 2007. Sexually Transmitted Disease Surveillance 2006. U.S. Department of Health and Human Services, November. Atlanta, GA.
3. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Carbohydrate fermentation plate medium for confirmation of Neisseria species. J. Clin. Microbiol. **1**:294-297.
4. Guide for the diagnosis of Gonorrhoea using culture and Gram stained smear. 1985. U.S. Department of Health and Human Services, Atlanta, GA.
5. **Ison, C. A., K. McLean, J. Gedney, P. E. Munday, D. Coghill, R. Smith, J. R. Harris, and C. S. Easmon.** 1985. Evaluation of a direct immunofluorescence test for diagnosing gonorrhoeae. J. Clin. Pathol. **38**:1142-1145.
6. **Kellogg, D. S., K. K. Holmes, and G. A. Hill.** 1976. Cumulative techniques and procedures in clinical microbiology: laboratory diagnosis of gonorrhoea, p. 1-10. In S. Marcus and J.C. Sherris (ed.) Cumitech 4, American Society for Microbiology, Washington D. C.
7. **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. Thornsberry, et al., (ed.) *Legionella*: proceedings of the 2nd international symposium, American Society for Microbiology, Washington, D. C.
8. **Martin, J. E. and J. S. Lewis.** 1977. Improved mycotic activity in modified Thayer-Martin medium. The Public Health Laboratory **35**:53.
9. **McCormack, W. M.** 1981. Clinical spectrum of infection with *Neisseria gonorrhoeae*. Sex. Trans. Dis. **8 (4 Suppl)**:305-307.
10. **Morello, J. A., W. M. Janda, and M. Bohnhoff.** 1985. *Neisseria* and *Branhamella*. p. 176-192. In E. H. Lennette, et al. (ed.), Manual of Clinical Microbiology, American Society for Microbiology, Washington, D. C.
11. **Morse, S. A., S. Stein, and J. Hines.** 1974. Glucose metabolism in *Neisseria gonorrhoeae*. J. Bacteriol. **120**:702-714.
12. **Panke, E. S., L. I. Yang, P. A. Leist, P. Magevney, R. J. Fry, and R. F. Lee.** 1991. Comparison of Gen-Probe DNA probe test and culture for the detection of *Neisseria gonorrhoeae* in endocervical specimens. J. Clin. Microbiol. **29**:883-888.
13. **Reddick, A.** 1975. A simple carbohydrate fermentation test for identification of pathogenic *Neisseria*. J. Clin. Microbiol. **2**:72-73.
14. **Sng, E. H., V. S. Rajan, K. L. Yeo, and A. J. Goh.** 1982. The recovery of *Neisseria gonorrhoeae* from clinical specimens: effects of different temperatures, transport time and media. Sex. Trans. Dis. **9**:74-78.
15. **Spengler, M. S., G. T. Rodeheaver, C. Richter, M. T. Edgerton, and R. F. Edlich.** 1978. The Gram stain, the most important diagnostic test infection. J. Am. Coll. Emerg. Phys. **7**:434-438.
16. **Thayer, J. D., and J. E. Martin, Jr.** 1966. Improved medium selection for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health Rep. **81**:559-562.

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