



AccuProbe[®]

NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST

Intended Use

The ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Neisseria gonorrhoeae* isolated from culture.

Summary and Explanation of the Test

Gonorrhea is the most commonly reported bacterial infection in the United States with nearly 767,000 cases reported in 1987. 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. While severe complications and sterility can occur in untreated individuals, asymptomatic infections are frequently diagnosed. Gonorrheal infections also may be diagnosed from other mucous membranes including the conjunctiva, anus and oropharynx (8).

Neisseria gonorrhoeae (*N. 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 identification of gonorrheal infections include fluorescent antibody staining, carbohydrate degradation, agglutination and sugar fermentation tests (2,4,10,11,13).

The ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST identifies *N. gonorrhoeae* isolated from culture within 30 minutes.

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 AccuProbe 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 Selection Reagent allows for the differentiation of non-hybridized and hybridized probe. The labeled DNA:RNA hybrids are measured in a Hologic luminometer. A positive result is a luminometer reading equal to or greater than the cut-off. A value below this cut-off is a negative result.

Reagents

Note: For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.

Reagents for the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE NEISSERIA GONORRHOEAE PROBE KIT

Probe Reagent (2 x 10 tubes).
Neisseria gonorrhoeae.

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT

Reagent 1 (Lysis Reagent). 1 x 10 mL
buffered solution containing 0.04% sodium azide.

Reagent 2 (Hybridization Buffer). 1 x 10 mL
buffered solution.

Reagent 3 (Selection Reagent). 1 x 60 mL
buffered solution.

HOLOGIC DETECTION REAGENT KIT

Detection Reagent I. 1 x 240 mL
0.1% hydrogen peroxide in 0.001 N nitric acid.

Detection Reagent II. 1 x 240 mL
1 N sodium hydroxide.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Use universal precautions when performing this assay (1).
- Use only for the identification of *N. gonorrhoeae* isolated from culture.
- Use only supplied or specified disposable laboratory ware.
- 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.
- 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.

Storage and Handling Requirements

Probe Reagent Tubes must be stored in the foil pouches at 2° to 8°C. The Probe 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 two months and prior to the expiration date.

Other reagents used in the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST may be stored between 2° to 25°C and are stable until the expiration date indicated.

DO NOT FREEZE THE REAGENTS.

Sample Collection and Preparation

The ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST is designed to determine the identity of *N. gonorrhoeae* isolated from culture.

Solid Media Method. Growth from appropriate solid media with morphology suggestive of *Neisseria gonorrhoeae* may be tested. Samples may be tested as soon as growth is visible but should be less than 48 hours old.

- Growth can be removed with a 1 µL disposable plastic loop, a wire loop, a disposable plastic needle or an applicator stick. Swabs should not be used due to the small volume of liquid in which the cells are subsequently resuspended.
- If a single colony is to be tested, it should be at least 1 mm in diameter. A 1µL loopful of cells or several (3 to 4) smaller colonies can be tested.

- C. Avoid taking any of the solid media with the cells.
- D. The operator may elect to inoculate another culture plate at this time to confirm the purity of the isolate.

Materials Provided

The ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST

Cat. No. 102830

Probe Reagent

20 Tests

2 x 10 tubes

Materials Required But Not Provided

1 µL plastic sterile inoculating loops, wire loops, plastic needles, or applicator sticks for selecting colonies.

Control culture strains

Water bath or dry heat bath* (60° ± 1° C)

Micropipettes (50 µL, 300 µL)

Re-pipettor (50 µL, 300 µL)

Vortex mixer

*Heating blocks in the dry heat bath should have wells that are correctly sized for 12 x 75 mm tubes. The use of Hologic dry heat baths is recommended.

AVAILABLE FROM HOLOGIC:

Hologic Leader® Luminometer

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT (Cat. No. 102800)

HOLOGIC DETECTION REAGENT KIT (Cat. No. 201791)

Hologic Dry Heat Bath (Cat. No. 102775)

Test Procedure

A. EQUIPMENT PREPARATION

1. Adjust the dry heat bath or water bath to 60° ± 1°C.
2. Prepare the Hologic luminometer for operation. Make sure there is sufficient volume of Detection Reagents I and II to complete the tests.

B. CONTROLS

Positive and negative control strains should be tested routinely in each laboratory according to local regulations. A culture of *N. gonorrhoeae* (e.g., American Type Culture Collection, ATCC #19424) may be used as the positive control while a culture of *Neisseria meningitidis* (e.g., ATCC #13077) may be used as the negative control.

C. SAMPLE PREPARATION

1. Open the foil pouch by cutting evenly across the top of the pouch. Remove enough Probe Reagent Tubes to test the culture isolates and/or 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. Label a sufficient number of Probe Reagent Tubes to test the culture isolates and/or controls. Remove and retain the caps.
3. Pipette 50 µL of Reagent 1 (Lysis Reagent) into all Probe Reagent Tubes.
4. Transfer the sample from the solid media into the labeled Probe Reagent Tubes as described in the SAMPLE COLLECTION AND PREPARATION Section. Twirl the loop, needle or stick in the Reagent 1 (Lysis Reagent) to remove the cells.

D. HYBRIDIZATION

1. Pipette 50 µL of Reagent 2 (Hybridization Buffer) into all Probe Reagent Tubes. Recap the Probe Reagent Tubes and mix by shaking or vortexing.

2. Incubate for 15 minutes at 60° ± 1°C in a water bath or dry heat bath.

E. SELECTION

1. Remove the Probe Reagent Tubes from the water bath or dry heat bath. Remove and retain the caps. Pipette 300 µL of Reagent 3 (Selection Reagent) into each tube. Recap the tubes and VORTEX them to mix completely.
2. Incubate the Probe Reagent Tubes for 5 minutes at 60° ± 1°C in a water bath or dry heat bath.
3. Remove the Probe Reagent Tubes from the water bath or dry heat bath and leave them at room temperature for at least 5 minutes. Remove and discard the caps. **Read the results in the luminometer within 30 minutes after removing from the water bath or dry heat bath.**

F. DETECTION

1. Select the appropriate protocol from the menu of the luminometer software.
2. Using a damp tissue or paper towel, wipe each tube to ensure that no residue is present on the outside of the tube and insert the tube into the luminometer according to the instrument directions.
3. When the analysis is complete, remove the tube(s) from the luminometer.

Procedural Notes

- A. REAGENT: Reagent 2 (Hybridization Buffer) may precipitate. Warming and mixing the solution at 35° to 60°C will dissolve the precipitate.
- B. TEMPERATURE: The Hybridization and Selection reactions are temperature dependent. Therefore, it is imperative that the water bath or dry heat bath is maintained within the specified temperature range.
- C. TIME: The Hybridization and Selection reactions are time dependent. Hybridize at least 15 minutes but no more than 20 minutes. Incubate the Probe Reagent Tubes during the SELECTION Step for at least 5 minutes but no more than 6 minutes.
- D. WATER BATH: The level of water in the water bath should be maintained to ensure that the entire liquid reaction volume in the Probe Reagent Tubes is submerged.
- E. VORTEXING: It is critical to have a homogeneous mixture during the SELECTION Step, specifically after the addition of Reagent 3.
- F. TROUBLE SHOOTING:
 1. Elevated negative control values (*Neisseria meningitidis* ATCC #13077) greater than 20,000 RLU (Relative Light Units) in the Leader or 600 PLU (Photometric Light Units) in the AccuLDR (formerly PAL) can be caused by insufficient mixing after adding Reagent 3 (Selection Reagent) or by testing mixed cultures. Because mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.
 2. Low positive control values (*N. gonorrhoeae*, ATCC #19424) less than 50,000 RLU in the Leader or 1,500 PLU in the AccuLDR (formerly PAL) can be caused by insufficient cell numbers or by testing mixed or aged cultures. Because mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.

Results

A. INTERPRETATION OF RESULTS

The results of the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST are based on the following cut-off values. Samples producing signals greater than or equal to these cut-off values are considered positive. Signals less than these cut-off values are considered negative. Results in repeat ranges should be repeated.

	AccuLDR (formerly PAL)	Leader
Cut-off value	1,500 PLU	50,000 RLU
Repeat range	1,200-1,499 PLU	40,000-49,999 RLU

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative control (e.g., *N. meningitidis*, ATCC #13077) and positive control (e.g., *N. gonorrhoeae*, ATCC # 19424) should satisfy the following values:

	AccuLDR (formerly PAL)	Leader
Negative control	< 600 PLU	< 20,000 RLU
Positive control	> 1,500 PLU	> 50,000 RLU

Limitations

This method has been tested using fresh growth from solid media mentioned in the SAMPLE COLLECTION AND PREPARATION Section. The efficacy of this test has not been demonstrated on direct clinical specimens.

Results from the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Expected Values

The ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST was compared to standard culture identification methods at three clinical sites using 308 isolates of *N. gonorrhoeae* and 239 other bacterial isolates representing 46 genera. Standard culture identification methods include Gram stain, oxidase reactions and a series of carbohydrate degradation tests or one of the commercially available identification methods. The isolates were categorized as either positive ($\geq 50,000$ RLU) or negative ($< 50,000$ RLU). The range of observations for negative cultures was 200 to 41,219 RLU and 56,741 to 1,072,977 RLU for positive cultures. A comparison of these results to standard culture identification methods is shown below.

ACCUPROBE CULTURE IDENTIFICATION

AccuProbe Culture	Pos Pos	Pos Neg	Neg Pos	Neg Neg	Sensitivity/ Specificity	Percent Agreement
Site 1	149	0	0	124	100%/100%	100%
Site 2	104	0	0	101	100%/100%	100%
Site 3	55	0	0	14	100%/100%	100%
Total	308	0	0	239	100%/100%	100%

Performance Characteristics

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST was calculated by assaying two concentrations of ribosomal RNA isolated from *N. gonorrhoeae* using 10 replicates in a single assay.

Sample	A	B
Number of Replicates	10	10
Mean Response	60,572	100,904
Standard Deviation	1,030	2,502
Coefficient of Variation	1.7%	2.5%

B. BETWEEN-RUN PRECISION

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

Sample	A	B
Number of Replicates	12	12
Mean Response	55,825	97,168
Standard Deviation	4,472	6,082
Coefficient of Variation	8.0%	6.3%

C. SPECIFICITY

A total of 155 *N. gonorrhoeae* and 231 other culture isolates were evaluated using the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST. These isolates represented a total of 79 species from 46 genera. Fifty-six isolates of 11 species of *Neisseria*, including *N. lactamica* and *N. meningitidis*, and 67 other species from 45 genera representing a phylogenetic cross-section of organisms were evaluated using the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST. Only those isolates of *Neisseria gonorrhoeae* produced a positive result.

D. RECOVERY

Neisseria meningitidis was added at concentrations of 10 million cells per test alone and to samples containing between 1 thousand and 10 million cells of *N. gonorrhoeae*. *N. meningitidis* did not react in the test or interfere with the recovery of *N. gonorrhoeae* using the ACCUPROBE NEISSERIA GONORRHOEAE CULTURE IDENTIFICATION TEST.

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102846 Rev. 001
2016-03