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DNA Probe Confirmatory Test for *Neisseria gonorrhoeae*

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A DNA probe test for the culture confirmation of *Neisseria gonorrhoeae* in clinical isolates was evaluated with 156 isolates of *N. gonorrhoeae* and 120 isolates of nongonococcal *Neisseria* species, organisms representative of other genera within the family *Neisseriaceae*, and organisms isolated on media selective for *N. gonorrhoeae*. The 30-min test used a chemiluminescent DNA probe that was homologous to rRNA sequences of *N. gonorrhoeae*. We report here a specificity and a sensitivity of 100% with the 276 clinical isolates tested, including 43 gonococcal strains that had been misidentified by other methods.

Neisseria gonorrhoeae is the causative agent of the most commonly reported bacterial infection in the United States; more than 689,000 cases of of gonorrhea were reported to the Centers for Disease Control in 1989 (2). Gonorrhea is a sexually transmitted disease which is manifested primarily as acute urethritis in males and most commonly as cervicitis in females, although the urethra may also be affected. *N. gonorrhoeae* commonly infects mucosal surfaces of the genitourinary tract but can also be isolated from other mucosal surfaces, including the oropharynx, rectum, and conjunctiva (5).

A presumptive diagnosis of *N. gonorrhoeae* is based upon the recovery of gram-negative, cytochrome oxidase-positive diplococci from cultures. Morphologically similar nonpathogenic organisms can be isolated from the same sites as *N. gonorrhoeae*. *N. gonorrhoeae* is frequently isolated from asymptomatic females. Accurate diagnosis in these individuals is critical because, if left untreated, gonorrhea can result in more serious complications (e.g., pelvic inflammatory disease) that can cause sequelae such as sterility or ectopic pregnancy (6). In special situations (e.g., infections involving prepubescent children), the diagnosis of gonorrhea may have serious medical, social, and legal ramifications. For these reasons, many laboratories perform more than one confirmatory test to establish a definitive diagnosis. Confirmatory methods currently available include fluorescent-antibody staining, coagglutination, carbohydrate utilization, and enzyme-based tests. Recent reports have emphasized the limitations of many of these tests (3, 4, 7).

We report here the results of an evaluation of a new DNA probe test to confirm the presence of *N. gonorrhoeae*. A total of 156 isolates of *N. gonorrhoeae* and 120 isolates of nongonococcal *Neisseria* species, organisms representative of other genera within the family *Neisseriaceae*, and organisms isolated on media selective for *N. gonorrhoeae* were tested. The *N. gonorrhoeae* isolates were chosen for their serologic (11 IA and 14 IB serovars and 25 nontypeable isolates) and geographic (isolates from 12 state health departments and 4 foreign countries) diversity. In addition, many isolates gave weak or negative reactions with the commercially available serologic tests used for the confirmation of *N. gonorrhoeae*. Forty-one isolates gave negative reactions with the monoclonal fluorescent-antibody test, and two gave negative reactions with the monoclonal antibody coagglutination test. Most of the nongonococcal isolates had been submitted to the Centers for Disease Control as diagnostic problem isolates for identification. Included among these isolates were those that gave positive reactions with serologic tests and those that gave biochemical test results that would lead to a misidentification as *N. gonorrhoeae*.

All of the organisms were subcultured onto either chocolate agar or selective gonococcal media and incubated overnight at 35°C in a humidified atmosphere supplemented with carbon dioxide. Testing was performed after 24 to 48 h of incubation. In some experiments, cultures were incubated for 96 to 120 h.

Isolates of *N. gonorrhoeae* were presumptively identified by standard procedures, i.e., Gram staining, oxidase reaction, and colony morphology. In addition, all isolates were confirmed by biochemical and serologic methods, including sugar utilization tests (Quadferm +; Analytab Products, Plainview, N.Y.), coagglutination (GonoGen II; New Horizons, Columbia, Md.), or fluorescent-antibody tests (Syva Co., Palo Alto, Calif.). All nongonococcal isolates were identified by relevant biochemical tests and any additional bacteriologic procedures as necessary (6). Confirmatory tests were performed in accordance with the package inserts provided by the manufacturers. Tests for nitrite reduction, polysaccharide production from 5% sucrose, and colistin susceptibility were performed to identify *N. cinerea* and *Branhamella catarrhalis*.

The AccuProbe *Neisseria gonorrhoeae* Culture Confirmation Test (Gen-Probe, Inc., San Diego, Calif.) was performed in accordance with manufacturer instructions, as follows. A 1-μl loopful of cells, a single colony at least 1 mm in diameter, or several (three to four) smaller colonies were transferred to reaction tubes containing 50 μl of reagent 1 (specimen diluent). Fifty microliters of reagent 2 (probe reagent) was added, and the tubes were incubated at 60°C for 15 min. After the addition of 300 μl of reagent 3 (selection reagent), the tubes were vortexed briefly, incubated for 5 min at 60°C, and read in a luminometer (leader I; Gen-Probe). The values were reported as relative light units (RLU). Samples with RLU of >50,000 were considered positive for the presence of *N. gonorrhoeae*, and samples with RLU of <50,000 were considered negative. This evaluation was performed with a prototype kit. The manufacturer has made two changes to the assay prior to marketing it. The composition of reagent 1 has been changed, and the chemiluminescent probe has been lyophilized in the individual reaction tubes to improve the shelf-life of the kit. We

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TABLE 1. Results of the AccuProbe Test for *N. gonorrhoeae*

Organism	No. of isolates tested[a]	RLU
Neisseria gonorrhoeae	156 (50)	83,518–1,672,407
Branhamella catarrhalis	13 (5)	474–2,322
Candida albicans	1	660
Kingella denitrificans	3	346–1,034
Neisseria cinerea	28 (10)	374–8,875
Neisseria flava	3	617–1,524
Neisseria flavescens	1	2,462
Neisseria lactamica	20 (10)	338–6,194
Neisseria meningitidis	30 (10)	275–3,859
Neisseria mucosa	4	1,019–2,918
Neisseria perflava	3	540–1,007
Neisseria polysaccharea	1	965
Neisseria sicca	6	493–2,892
Neisseria subflava	5	895–4,576
Neisseria species	1	495
Staphylococcus aureus	1	560

[a] Numbers in parentheses represent the numbers of isolates tested with the new test format.

tested a subset of isolates with the new format and found that these changes did not affect the RLU obtained or test performance.

The results are summarized in Table 1. The specificity of the DNA probe test was 100%. No positive reactions were observed with any of the nongonococcal isolates tested. The values ranged from a low reading of 275 RLU with the closely related *N. meningitidis* to a high reading of 8,875 RLU with a strain of *N. cinerea*. All of the readings were significantly below the cutoff value of 50,000 RLU. The sensitivity of the test was also 100%. All 156 strains of *N. gonorrhoeae* tested after 48 h of incubation gave strongly positive results. The mean value was 1,086,068 RLU, with a range of 83,518 to 1,672,407. Thus, most of the gonococcal isolates gave readings that were 2 to 3 orders of magnitude greater than those of the nongonococcal isolates tested. To minimize the effects of endogenous RNase activity, the manufacturer recommends that cultures be incubated for no more than 24 h. However, it is not always possible to have 24-h cultures available for testing. Therefore, we investigated the effects of incubation time on chemiluminescence. Five isolates belonging to either serovar IA-1 or serovar IB-1 were incubated for 24, 96, or 120 h and tested. After 96 h of incubation, there was a 43 to 71% decrease in the RLU. However, all of the RLU obtained were considerably above the cutoff value of 50,000 RLU. The chemiluminescence values for some isolates incubated for 120 h were, however, below the positive cutoff value of 50,000 RLU.

The AccuProbe Test uses a single-stranded DNA probe that is complementary to unique rRNA sequences of *N. gonorrhoeae*, the target organism. The probe is labeled with a chemiluminescent acridinium ester. Differentiation between the hybridized probe and the nonhybridized probe is based on the differential hydrolysis of the ester bond in the hybridized and nonhybridized acridinium ester-labeled probes (hybrid protection assay). The label is protected from hydrolysis when the probe is hybridized to its target RNA, whereas hydrolysis is accelerated when the probe is not hybridized. After differential hydrolysis, the remaining chemiluminescence is a direct measure of the amount of hybrid formed (1).

The AccuProbe Test confirms primary isolates in less than 30 min with only 5 to 10 min of hands-on time. This study has demonstrated that the AccuProbe Test has excellent specificity and sensitivity. This DNA probe-based test provides a practical and rapid alternative to any confirmatory test which requires subculturing. In addition, this test eliminates the problems now encountered in attempts to provide a definitive identification of *N. gonorrhoeae*.

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