

Comparative Evaluation of AccuProbe Culture Identification Test for *Neisseria gonorrhoeae* and Other Rapid Methods

HUGH YOUNG* AND ALEX MOYES

Scottish Gonococcal Reference Laboratory, Department of Medical Microbiology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland

Received 19 January 1993/Accepted 4 May 1993

The AccuProbe chemiluminescent culture identification test for *Neisseria gonorrhoeae* (Gen-Probe Inc., San Diego, Calif.) was assessed in a comparative evaluation with other rapid methods by using 269 isolates of oxidase-positive, gram-negative diplococci. Chemiluminescence was read with a PAL luminometer, and results were expressed as PAL light units (PLUs): the cutoff value for a positive identification was 1,500 PLUs. All 200 isolates of gonococci confirmed by carbohydrate utilization and serotyped with monoclonal antibodies were identified correctly by AccuProbe on initial testing. The API Quadferm system (Bio Merieux, Marcy l'Etoile, France) identified 95% ($n = 190$) of the gonococci correctly on initial testing and 99.5% ($n = 199$) on repeat testing, while the Phadebact Monoclonal GC test (Kara Bio Diagnostics AB, Huddinge, Sweden) identified 95.5% ($n = 191$) of the gonococci on both initial and repeat testing; 8 of the Phadebact-negative isolates were all of the same rare serovar (serovar 1B-17). The mean PLU for the gonococcal isolates was 9,014 (range 2,264 to 10,845) compared with a mean of 51 (range, 8 to 109) for the 69 nongonococcal isolates. We conclude that the AccuProbe culture confirmation test provides a rapid and accurate objective means of identifying cultured *N. gonorrhoeae* isolates.

Several commercial identification systems based on a variety of biochemical and immunological methods are available for the rapid identification of *Neisseria gonorrhoeae* (3). Recently, DNA probe technology has created new diagnostic approaches for use in the clinical laboratory (8, 20, 23). Several tests have now been developed for the direct detection of target sequences of *N. gonorrhoeae* either in patient exudates (5, 6, 13, 18, 19) as a means of noncultural diagnosis or in cultured organisms (1, 11, 12, 16, 22) to provide confirmatory identification. Probes based on target sequences of chromosomal DNA have been evaluated most extensively as identification tests, but with a few exceptions (11), they have given problems with specificity (1, 16, 22) and sensitivity (22). DNA probes can also be derived from rRNA (4), and this approach has been used in the AccuProbe culture identification test for *N. gonorrhoeae* (Gen-Probe Inc., San Diego, Calif.). The AccuProbe test uses a chemiluminescent labelled single-stranded DNA probe that is complementary to gonococcal rRNA. Test bacteria are lysed to release the rRNA, which, in the case of gonococci, combines with the DNA probe to form a stable DNA-RNA hybrid. A selection reagent differentiates between the nonhybridized and the hybridized probes. The labelled DNA-RNA hybrids are measured in a Gen-Probe luminometer, and the results are expressed as relative light units (RLUs). A prototype AccuProbe identification test demonstrated 100% sensitivity and specificity (12). We report a comparative evaluation of a commercially available Gen-Probe kit with other rapid methods for the identification of *N. gonorrhoeae*.

MATERIALS AND METHODS

A total of 269 isolates of oxidase-positive, gram-negative diplococci were included in the study. These comprised strains submitted to the Scottish Gonococcal Reference Laboratory for gonococcal serotyping and antibiotic suscep-

tibility testing, unselected oxidase-positive, gram-negative diplococci isolated in our laboratory from cultures of anogenital and throat specimens from patients undergoing screening for gonococcal infection or from cultures of throat swabs that were requested for routine culture, and a strain of *Neisseria cinerea* from the National Collection of Type Cultures (NCTC 10294). All isolates were subcultured onto modified New York City medium (25) lacking selective antibiotics, incubated overnight at 37°C, and used as the inoculum for the following identification procedures, which were performed in parallel.

The rapid carbohydrate utilization test (RCUT) was performed by using in-house reagents as described previously (25, 28), but microwell strips rather than individual tubes were used. Each strip included wells for glucose, maltose, sucrose, lactose, fructose, and ampicillin in a buffer containing phenol red pH indicator. Results were read after 3 h of incubation at 37°C. The Phadebact Monoclonal GC test (Kara Bio Diagnostics AB, Huddinge, Sweden) was performed according to the manufacturer's instructions. This coagglutination test uses two reagents comprising pools of murine monoclonal antibodies that are reactive with epitopes on isolates with proteins IA (WI reagent) and IB (WII/III reagent), respectively. A boiled suspension of the test organism was tested against each reagent, and results were read within 1 min as described previously (26). The API Quadferm system (Bio Merieux, Marcy-l'Etoile, France) is a standardized system for the rapid identification of *Neisseria* and *Branhamella* species and the determination of penicillinase activity. Each test strip contains seven microcupules comprising a control well and glucose, maltose, lactose, sucrose, DNase, and penicillinase test wells.

Test strips were inoculated, incubated, and read after 2 h according to the manufacturer's instructions. The AccuProbe *N. gonorrhoeae* culture confirmation test (Gen-Probe) was performed according to the manufacturer's instructions. For each specimen, a 1- μ l loopful of cells or several (three to four) small colonies was transferred to a probe reagent tube

* Corresponding author.

TABLE 1. Identification test results for 269 isolates of gram-negative diplococci

Method	No. of isolates			
	Positive, <i>N. gonorrhoeae</i>		Negative, nongonococcal	
	First test	Retest	First test	Retest
AccuProbe	200		69	
Phadebact	191	191	78	78
Quadferm	190	199	79	70
RCUT	200		69	

containing 50 μ l of lysis reagent; the loop was twirled in the lysis reagent to remove the cells. Hybridization reagent (50 μ l) was then added and the tubes were capped, mixed by vortexing, and incubated for 15 min at 60°C. After the addition of 300 μ l of selection reagent, the tubes were vortexed, incubated for 5 min at 60°C, and read in a PAL/AccuLDR luminometer (Gen-Probe). The values were reported as PAL light units (PLUs). Samples with PLUs of >1,500 were considered positive, those with PLUs of 1,200 to 1,499 were considered equivocal, and those with PLUs of <1,200 were considered negative. Serotyping of suspect gonococcal isolates was performed as described previously (15) by using the Genetic Systems panel of monoclonal antibodies (9). Strains that were nontypeable with this panel were typed with the Pharmacia panel of monoclonal antibodies (15).

RESULTS

The results of testing of the 269 isolates are summarized in Table 1. Because the Phadebact and AccuProbe tests can differentiate only between gonococci and nongonococcal organisms, the actual identity of each nongonococcal isolate is not given. The 200 isolates that gave positive reactions by the AccuProbe and RCUT methods were confirmed as *N. gonorrhoeae* with monoclonal antibodies. The isolates included 68 serovar IA strains (34 IA-2, 3 IA-4, 3 IA-5, 5 IA-6, 17 IA-16, 4 IA-21, 2 IA-25) and 132 serovar IB strains (24 IB-1, 26 IB-2, 14 IB-3, 3 IB-5, 12 IB-6, 8 IB-7, 4 IB-8, 3 IB-15, 3 IB-16, 8 IB-17, 3 IB-19, 1 IB-24, 5 IB-26, 2 IB-29, 3 IB-31, and 13 nontypeable with Genetic Systems reagents but serovar Bx/Av with Pharmacia reagents). The mean PLU for the 200 gonococcal isolates was 9,014 (range, 2,264 to 10,845). Of the 10 gonococcal isolates not identified correctly by Quadferm on initial testing, 6 were identified as *Neisseria meningitidis*, 2 as *Moraxella catarrhalis*, 1 as the *Neisseria sicca-Neisseria subflava-Neisseria mucosa* group, and 1 no identity. On retesting, nine isolates were identified correctly as *N. gonorrhoeae*, while the strain identified initially as a member of the *N. sicca-N. subflava-N. mucosa* group gave the same identity. All nine Phadebact-negative gonococcal isolates remained negative on repeat testing; eight isolates were serovar IB-17 and 1 isolate was serovar IA-16. Because no nongonococcal organisms were identified as *N. gonorrhoeae*, the positive predictive value for all four tests with respect to identifying gonococci was 100%. The negative predictive value with respect to identifying an isolate as nongonococcal was also 100% for AccuProbe and RCUT. The negative predictive value for Quadferm was 87.4% on the first test (10 false-positive nongonococcal identifications) and 98.6% on repeat testing (one false-positive nongonococcal identification). The negative predictive

value for Phadebact was 88.5% for both initial and repeat testing (nine false-positive nongonococcal identifications).

The mean PLU was 51 (range, 8 to 109) for the 69 nongonococcal isolates. The RCUT identified the 69 nongonococcal isolates as *N. meningitidis* ($n = 22$), *Neisseria lactamica* ($n = 20$), *Neisseria perflava* ($n = 5$), and *M. catarrhalis* ($n = 22$); the *N. cinerea* isolate was wrongly identified as *M. catarrhalis*. The mean PLUs for the various organisms were 46.4 for *N. meningitidis*, 49.5 for *N. lactamica*, 52.6 for *N. perflava*, 56.6 for *M. catarrhalis*, and 42 for one isolate of *N. cinerea*. Quadferm results agreed with the RCUT results for all of the *N. meningitidis* and *N. lactamica* isolates and for 21 of the *M. catarrhalis* isolates; no identification was obtained for the discordant isolate with Quadferm. Because additional culture tests were not undertaken, four of the five *N. perflava* isolates were identified within the *N. sicca-N. subflava-N. mucosa* group; the fifth isolate was identified as *M. catarrhalis*. No identification was obtained for the strain of *N. cinerea* with Quadferm (*N. cinerea* is not listed in the Quadferm identification table).

DISCUSSION

Not only did the AccuProbe test give 100% sensitivity and specificity but it also showed excellent discrimination between gonococci and nongonococcal organisms with respect to PLUs. No isolate gave a PLU result in the equivocal range. The mean PLU for gonococci was 6 times (range, 1.5 to 7.2 times) the cutoff value (1,500) for a positive result read on the PAL luminometer. The cutoff value (1,200) for a negative result was 23.5 times (range, 11 to 150 times) the mean PLU for nongonococcal organisms. These results confirm the 100% sensitivity and specificity found in the evaluation of the prototype AccuProbe kit by using the Leader I luminometer (12). The differentiation was, however, slightly greater in the latter study; the mean RLU for gonococci was 21.7 times (range, 1.7 to 33.4 times) above the cutoff (50,000) for a positive result, while values for nongonococcal organisms ranged from 181.8 times (for a strain of *N. meningitidis*) to 5.6 times (for a strain of *N. cinerea*) below the cutoff. Harada et al. (7) reported a significant correlation between the AccuProbe result in terms of RLUs and the number of gonococci ($r = 0.96$); the threshold of detection was 5×10^3 CFU per tube. The sensitivity of detection of gonococci was not influenced by the presence of other bacteria (7), although endogenous RNase activity may decrease the RLU by 43 to 71% when cultures are incubated for 96 h (12). This decrease did not result in a decrease in the RLUs given by any of the gonococci below the cutoff. Nevertheless, the manufacturer recommends that colonies should be less than 48 h old.

Because the strains evaluated in the present study include isolates that were negative by other test systems, the absolute sensitivity of AccuProbe is particularly reassuring. Serovar IB-17 has previously been reported to be negative in the Phadebact Monoclonal GC test (26). However, because the prevalence of this serovar is low in most geographical areas (14, 27), the performance of the Phadebact Monoclonal GC test is generally in excess of 99% rather than 95.5% as reported here. The reason for the poor initial identification of *N. gonorrhoeae* by Quadferm is unclear, because all nine gonococcal isolates that were misidentified initially were identified correctly on repeat testing. Although it was not used in the present study, the Syva Micro Trak fluorescent-antibody identification test was negative for the very rare serovar IB-24 (14). Two penicillinase-producing isolates of

N. gonorrhoeae serovar IB-19 were also found to be negative by the Syva test (24), even though a high degree of sensitivity was obtained with serovar IB-19 during initial trials.

The absolute specificity of AccuProbe is also reassuring because of difficulties that have been encountered in differentiating between *N. cinerea* and *N. gonorrhoeae* (2, 10). In one case, this resulted in proctitis associated with *N. cinerea* being misdiagnosed as a gonococcal infection in an 8-year-old boy (4). The failure of some strains of meningococci to give a positive reaction in certain biochemical test systems (21) has resulted in colonization with "maltose-negative" meningococci being misdiagnosed as pharyngeal gonorrhoea (17). The high sensitivity and specificity of AccuProbe suggest that the same test principle may be of considerable value in detecting gonococci in patient exudates. Preliminary reports of direct detection in high-prevalence (21 to 24%) populations with the Gen-Probe PACE assay gave a sensitivity of approximately 90 to 93% and a specificity of 99% (5, 6). In a comparison of Gen-Probe PACE 2 with transported Gen-Probe swabs and conventional culture based on JEM-BEC plates incubated overnight prior to spending a minimum of 2 days in the mail system, 2.7% of specimens were positive by the probe method and 0.6% were positive by culture; indirect evidence suggested that the probe-positive, culture-negative specimens were false-negative cultures (13). Evaluations involving optimal culture procedures and low-prevalence populations are, however, lacking. The limit of detection of 5×10^3 CFU per tube by AccuProbe is severalfold in excess of the mean number of gonococci found in cervical aspirates (1×10^6 CFU/ml) and is similar to the minimum of the culture-positive range (5×10^3 to 8×10^6) (29). This supports the probability of good sensitivity, provided that an adequate sample is obtained.

We conclude that the AccuProbe culture confirmation test provides a rapid (total time, 30 min, with 5 to 10 min of hands-on time) and accurate objective means of identifying cultured *N. gonorrhoeae*. We have not evaluated the test using primary cultures, but the finding of Harada et al. (7) that detection sensitivity was not influenced by the presence of other bacteria suggests that this procedure should give similar results. Like immunological test systems, the probe test does not characterize nongonococcal neisserial isolates to the species level. We do not consider this to be an important factor in test selection in those laboratories whose main aim is to detect or exclude patients with gonococcal infections.

REFERENCES

- Bollet, C., M. Grandguillot, H. Bouchemal, N. Boutefnouchet, and P. de Micco. 1989. Evaluation of a biotinylated probe for identification of *Neisseria gonorrhoeae*. *Pathol. Biol. (Paris)* 37:897-900.
- Boyce, J. M., and E. B. Mitchell, Jr. 1985. Difficulties in differentiating *Neisseria cinerea* from *Neisseria gonorrhoeae* in rapid systems used for identifying pathogenic *Neisseria* species. *J. Clin. Microbiol.* 22:731-734.
- Dillon, J. R., M. Carballo, and M. Pauze. 1988. Evaluation of eight methods for identification of pathogenic *Neisseria* species: Neisseria-Kwik, RIM-N, Gonobio-Test, Minitek, Gonocheck II, GonoGen, Phadebact Monoclonal GC OMNI Test, and Syva Micro Trak Test. *J. Clin. Microbiol.* 26:493-497.
- Dossett, J. H., P. C. Appelbaum, J. S. Knapp, and P. A. Totten. 1985. Proctitis associated with *Neisseria cinerea* misidentified as *Neisseria gonorrhoeae* in a child. *J. Clin. Microbiol.* 21:575-577.
- Granato, P. A., and M. R. Franz. 1989. Evaluation of a prototype DNA probe test for the noncultural diagnosis of gonorrhoea. *J. Clin. Microbiol.* 27:632-635.
- Granato, P. A., and M. R. Franz. 1990. Use of the Gen-Probe PACE system for the detection of *Neisseria gonorrhoeae* in urogenital samples. *Diagn. Microbiol. Infect. Dis.* 13:217-221.
- Harada, Y., S. Miyazaki, Y. Kaneko, and S. Goto. 1991. A novel approach to the detection of *Neisseria gonorrhoeae* employing DNA probe method. *Kansenshogaku Zasshi* 65:844-850.
- Horn, J. E., T. Quinn, M. Hammer, L. Palmer, and S. Falkow. 1986. Use of nucleic acid probes for the detection of sexually transmitted infectious agents. *Diagn. Microbiol. Infect. Dis.* 4:101S-109S.
- Knapp, J. S., M. R. Tam, R. C. Nowinski, K. K. Holmes, and E. G. Sandstrom. 1984. Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein I. *J. Infect. Dis.* 150:44-48.
- Knapp, J. S., P. A. Totten, M. H. Mulks, and B. M. Minshew. 1984. Characterization of *Neisseria cinerea*, a nonpathogenic species isolated on Martin-Lewis medium selective for pathogenic *Neisseria* spp. *J. Clin. Microbiol.* 19:63-67.
- Kuritz, A. P., S. C. Edberg, C. Chapis, and P. Gallo. 1989. Identification of *Neisseria gonorrhoeae* with the ORTHOProbe DNA test. *Diagn. Microbiol. Infect. Dis.* 12:129-132.
- Lewis, J. S., D. Kranig Brown, and D. A. Trainor. 1990. DNA probe confirmatory test for *Neisseria gonorrhoeae*. *J. Clin. Microbiol.* 28:2349-2350.
- Limberger, R. J., R. Biega, A. Evancoe, L. McCarthy, L. Sliwinski, and M. Kirkwood. 1992. Evaluation of culture and the Gen-Probe PACE 2 assay for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in endocervical specimens transported to a state health laboratory. *J. Clin. Microbiol.* 30:1162-1166.
- Moyes, A., and H. Young. 1989. Fluorescent monoclonal antibody test for the confirmation of *Neisseria gonorrhoeae*. *Med. Lab. Sci.* 46:6-10.
- Moyes, A., and H. Young. 1991. Epidemiological typing of *Neisseria gonorrhoeae*: a comparative analysis of three monoclonal antibody serotyping panels. *Eur. J. Epidemiol.* 7:311-319.
- Naher, H., P. K. Kohl, and D. Petzoldt. 1989. Evaluation of a non-radioactive DNA probe for confirmatory identification of *Neisseria gonorrhoeae*. *Int. J. Med. Microbiol.* 272:181-185.
- Noble, R. C., and R. M. Cooper. 1979. Meningococcal colonization misdiagnosed as gonococcal pharyngeal infection. *Br. J. Vener. Dis.* 55:336-339.
- Palomares, J. C., M. C. Lozano, J. Aznar, and E. J. Perea. 1989. In situ hybridization with DNA probe for the diagnosis of *Neisseria gonorrhoeae* infections. *Enferm. Infecc. Microbiol. Clin.* 7:140-143.
- 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.
- Rossau, R., E. Vanmechelen, J. De Ley, and H. Van Heuverswijn. 1989. Specific *Neisseria gonorrhoeae* DNA-probes derived from ribosomal RNA. *J. Gen. Microbiol.* 135:1735-1745.
- Saez-Nieto, J. A., A. Fenoll, J. Vazquez, and J. Casal. 1982. Prevalence of maltose-negative *Neisseria meningitidis* variants during an epidemic period in Spain. *J. Clin. Microbiol.* 15:78-81.
- Sprott, M. S., A. M. Kearns, and M. W. Neale. 1989. Non-radioactive DNA probe to identify *Neisseria gonorrhoeae*. *Genitourin. Med.* 65:60-61.
- Tompkins, L. S. 1985. Recombinant DNA and other specimen identification techniques. *Clin. Lab. Med.* 5:99-107.
- Walton, D. T. 1989. Fluorescent-antibody-negative penicillinase-producing *Neisseria gonorrhoeae*. *J. Clin. Microbiol.* 27:1885-1886.
- Young, H. 1978. Identification and penicillinase testing of *Neisseria gonorrhoeae* from primary isolation cultures on modified New York City medium. *J. Clin. Microbiol.* 54:36-40.

26. **Young, H., and A. Moyes.** 1989. Utility of monoclonal antibody coagglutination to identify *Neisseria gonorrhoeae*. *Genitourin. Med.* **65**:8–13.
27. **Young, H., A. Moyes, D. H. H. Robertson, A. C. McCartney, G. Lindsay, G. Gallacher, I. B. Tait, O. Brogan, C. Fox, G. A. Kohiyar, D. Parratt, and I. Alexander.** 1990. Gonococcal infection within Scotland: antigenic heterogeneity and antibiotic susceptibility of infecting strains. *Eur. J. Epidemiol.* **6**:1–8.
28. **Young, H., I. C. Paterson, and D. R. McDonald.** 1976. Rapid carbohydrate utilisation test for the identification of *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.* **52**:172–175.
29. **Young, H., S. K. Sarafian, A. B. Harris, and A. McMillan.** 1983. Non-cultural detection of *Neisseria gonorrhoeae* in cervical and vaginal washings. *J. Med. Microbiol.* **16**:183–191.