Value of a DNA Probe Assay (Gen-Probe) Compared with That of Culture for Diagnosis of Gonococcal Infection

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The Gen-Probe PACE 2 system for *Neisseria gonorrhoeae* (GP), which uses a chemiluminescently labeled DNA probe, was compared with conventional culture as the method of reference. A total of 1,750 specimens were collected from 496 females and 623 males visiting the outpatient clinic of the Sexually Transmitted Diseases Department of the Westeinde Hospital, The Hague, The Netherlands, during the year 1991. The prevalences of gonorrhea culture-positive men and women were 14.9 and 7.7%, respectively. The overall positive rate was 8.7%. Sensitivity, specificity, and positive and negative predictive values of GP were 97.1, 99.1, 90.6, and 99.8%, respectively. A total of 12 of 13 patients with positive GP results and negative cultures may have had a gonococcal infection, a conclusion based on clinical symptoms, positive methylene blue smears, and high relative light unit ratios. The DNA probe test can be useful as a suitable screening and diagnostic test for gonorrheal infection in men and women. An advantage of using this DNA probe technique is that simultaneous testing for *Chlamydia trachomatis* of the same specimen is possible. We also examined whether (all) rRNA had disappeared after adequate treatment for gonococcal and/or chlamydial infection in 30 patients. None of those positive patients showed a positive result in the DNA probe assay after treatment.

Gonorrhea remains one of the most frequently occurring sexually transmitted diseases worldwide, with 3,666 cases reported in The Netherlands in 1990 (2). The control of epidemic infections and their sequelae remains dependent on an accurate and timely diagnosis. Noncultural techniques for diagnosing sexually transmitted diseases and, in particular, chlamydial and gonorrheal infections are now widely used. Such techniques are largely based on either immunofluorescence microscopy, enzyme immunoassay (1, 3, 12, 14), or nucleic acid hybridization (4, 10, 11, 15). These nonculture methods offer several advantages over conventional culture procedures in that the diagnosis is not dependent on the presence of viable microorganisms for microbial isolation and that turnaround times can be significantly reduced. However, none of these methods is completely reliable, and discrepancies with culture may occur (6, 7). Even culture does not detect all active infections (13), but remains the "gold standard" with which nonculture methods are compared. In this study, we describe the diagnostic value of the Gen-Probe DNA probe assay (PACE 2; Gen-Probe, Inc., San Diego, Calif.) for detection of *Neisseria gonorrhoeae* directly in specimens compared with conventional culture.

MATERIALS AND METHODS

Patients. The 1,750 specimens tested were collected from 496 females and 623 males attending the sexually transmitted diseases outpatient clinic of the Westeinde Hospital, The Hague, The Netherlands. All the patients were seen between January 1991 and January 1992.

Specimens. Cervical samples were taken after thorough removal of excess mucus and exudate with a Dacron swab. Subsequently, two endocervical samples were collected, one for the culture method and one for the DNA probe assay. For the urethral specimens from males, the same procedures were followed without cleansing. For routine bacterial cultures, the first swab taken was inoculated onto two solid media, Thayer-Martin agar (Oxoid) and New York City agar (Oxoid), and was examined after 24 h at 37°C (CO_2, 5%). Negative cultures were further incubated for up to 72 h. Isolates were identified as *N. gonorrhoeae* on the basis of growth and colony characteristics, Gram stain morphotype, and oxidase and catalase reactions and were confirmed with the Gen-Probe Accuprobe *N. gonorrhoeae* culture identification test (Gen-Probe, Inc.) (8). For each set of specimens collected, the Dacron swab used for the DNA probe assay was taken last. This last swab was placed in the Gen-Probe transport medium and was assayed the same day. A total of 1,750 specimens were collected from the urethra ($n = 1,076$), the cervix ($n = 491$), the pharynx ($n = 137$), and the rectum ($n = 46$).

DNA probe. The Gen-Probe PACE 2 *Chlamydia trachomatis*-*N. gonorrhoeae* Rapid Diagnostic System (GP) was used in this study. The PACE 2 system uses a single-stranded DNA probe, labeled with an acridinium ester that is complementary to the rRNA of the target organism (10, 11). All specimens were placed in the GP transport system and delivered to the laboratory and were assayed the same day. During transport, the rRNA was released from the organisms in the transport medium. A portion of the specimen transport medium was added to the DNA probe specific for either *C. trachomatis* or *N. gonorrhoeae* to form a stable DNA-RNA hybrid. The remaining labeled, nonhybridized probe was separated from the hybridized DNA probe by using magnetic particles in combination with a hydrolysis reagent (selection reagent). The selectivity of the selection reagent destroyed the chemiluminescent label of nonhybridized probe. The hybrids formed were not affected by this selection reagent and therefore retained their chemiluminescent properties. The adsorbed hybrids bound to the magnetic particles were separated by using a Gen-Probe magnetic

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TABLE 1. Comparison of culture and DNA probe assay for
N. gonorrhoeae detection[a]

DNA probe result	No. of cultures with the following result:		Total
	+	−	
+	135	14	149
−	4	1,597	1,601
Total	139	1,611	1,750

[a] DNA probe performance: sensitivity, 97.1%; specificity, 99.1% versus culture; positivity rate: 8.7%; predictive values: positive, 90.6%, and negative, 99.8%; prevalence: 11.9%.

separation unit. Finally, the tubes were washed and the labeled DNA-RNA hybrids were measured in a chemoluminometer, Leader I. The test results were calculated on the basis of the difference between the response of the specimen in relative light units (RLU) and the mean RLU of the negative reference. If this difference, expressed as RLU, exceeded the mean of the negative references by 300 RLU (cutoff), the sample was considered positive. The RLU ratio of sample RLU/cutoff RLU of ≥1.0 was considered positive as recommended by the manufacturer.

RESULTS

Of the 1,750 specimens tested, 139 were positive by culture methods. The probe assay detected 135 (115 patients) of the 139 culture positives, resulting in four false negatives. There were 14 false positives in the probe assay. Findings of culture and DNA probes are stated and compared in Table 1, resulting in sensitivity, specificity, and positive and negative predictive values of 97.1, 99.1, 90.6, and 99.8%, respectively, in a population with a gonococcal disease prevalence of 11.9%. Because the Gen-Probe test has not been licensed in the United States for use with pharyngeal and rectal specimens, we subdivided the samples according to specimen type; samples were also divided by patient gender (Table 2). The sensitivity, specificity, and positive and negative predictive values for genital specimens only were 97.0, 99.1, 90.8, and 99.7%, respectively (Table 2). The distribution of samples by specimen type, patient gender, and test result is shown in Table 3. On the basis of these data, the sensitivities, specificities, and positive and negative predictive values for genital and nongenital samples from the total population were calculated (Table 2). Overall, the probe assay compared favorably with culture for all specimen types tested and for specimens collected from males and females. Although relatively low numbers of positive specimens were obtained from nongenital sites, the probe assay showed good results. The sensitivity, specificity, and positive and negative predictive values for female specimens

TABLE 2. Sensitivity, specificity, and predictive values for DNA
probe assay versus culture as the gold standard

Sample type	No. of samples	% Sensitivity	% Specificity	Predictive value (%)	
				Positive	Negative
Female	1,069	94.2	99.3	87.5	99.6
Male	681	98.8	98.8	92.5	99.8
Genital	1,567	97.0	99.1	90.8	99.7
Nongenital	183	100.0	99.4	87.5	100.0
Total	1,750	97.1	99.1	90.6	99.8

TABLE 3. Distribution of samples by specimen type, patient
gender, and test result

Sample type	Urethral		Cervical (female)	Pharyngeal		Rectal	
	Female	Male		Female	Male	Female	Male
Positive by:							
Culture and probe[a]	24	83	21	4	1	0	2
Culture only[b]	2	1	1	0	0	0	0
Probe only[c]	3	6	4	0	1	0	0
Negative	424	533	465	119	12	2	42
Total	453	623	491	123	14	2	44

[a] Patients, n = 30 female and 85 male; specimens, n = 135.
[b] Patients, n = 3 female and 1 male; specimens, n = 4.
[c] Patients, n = 6 female and 7 male; specimens, n = 14.

were 100% (4 of 4), 100% (119 of 119), 100% (4 of 4), and 100% (119 of 119), respectively.

For male nongenital specimens, these same values were 100% (3 of 3), 98.2% (54 of 55), 75% (3 of 4), and 100% (54 of 54), respectively. The ratios of sample RLU to cutoff RLU were higher than 2.0 in seven out of eight positive samples derived from nongenital sites. In one sample (pharynx), the ratio was 1.6. For the 14 false-positive probe specimens, patient clinical information is stated in Table 4. Six male patients (no. 2, 5, 6, 9, 11, and 13) had urethral discharge, and one male patient (no. 7) had a painful throat caused by tonsillitis. In five of the male patients with urethral discharge (no. 5, 6, 9, 11, and 13), intracellular diplococci were observed by methylene blue staining of the discharge. Three female patients (no. 1, 3, and 10) complained of vaginal discharges, and three asymptomatic female patients (no. 4, 8, and 12) had previous gonorrheal infections or were at high risk because of promiscuous behavior and/or prostitution. The ratios of sample RLU to cutoff RLU ranged from 1.3 to 988.0. The distribution of sample RLU/cutoff RLU ratios for all samples tested is given as follows. Of the 1,597 specimens that were negative in the probe assay, over 99% had ratios of less than 0.5; only 11 negative specimens had ratios equal to or higher than 0.5. Of the 153 specimens which were shown to be positive by culture, probe assay, or both, 143 specimens (93.5%) had ratios greater than or equal to 2.0. Only six specimens (3.9%) had ratios of 1.0 to 2.0.

To determine the performance of the Gen-Probe assay in testing whether patients were cured, we collected specimens from 30 previously probe-positive patients after treatment for their gonorrheal (9 patients) or chlamydial (21 patients) infections. A total of 14 test specimens (seven urethral, six cervical, and one pharyngeal), which were collected from nine (six female and three male) patients diagnosed with gonorrhea, turned out to be negative (mean RLU ratio = 0.2). A total of 21 patients (16 females and 5 males), who originally tested positive for C. trachomatis with the probe assay, were all negative (mean RLU ratio = 0.1) with the probe assay after treatment (19 urethral and 11 cervical specimens).

DISCUSSION

Infection by N. gonorrhoeae remains an important sexually transmitted disease in sexually active adults. Culture on selective media is currently considered the gold standard. Successful culture requires organism viability and timely transport under stringent conditions to the laboratory for processing. Even under these conditions, the sensitivity is reported to be only 85 to 95% (13). DNA hybridization

TABLE 4. Clinical and epidemiological information for patients with false-positive probe results

Patient no.	Sex	Age (yr)	Specimen type	Previous gonorrhea infection	Promiscuity/ prostitution	Complaints	Methylene blue stain result[a]	RLU ratio[b]
1	F	21	Cervical	+		+	ND[c]	2.4
2	M	20	Urethral			+	ND	13.3
3	F	19	Cervical			+	ND	16.8
4	F	42	Urethral	+			ND	22.0
5	M	34	Urethral			+	+	387.0
6	M	27	Urethral			+	+	352.0
7	M	54	Pharyngeal			+	ND	3.8
8	F	21	Cervical		+		ND	1.3
8	F	21	Urethral		+		ND	41.1
9	M	21	Urethral			+	+	142.0
10	F	18	Urethral		+	+	ND	4.4
11	M	23	Urethral			+	+	586.0
12	F	37	Cervical	+	+		ND	1.4
13	M	22	Urethral		+	+	+	988.0

[a] Urethral discharge was stained with methylene blue.
[b] Sample RLU/cutoff RLU ratio.
[c] ND, not done.

technology provides an alternative identification method that offers rapid (2-h) results, as well as ease of specimen storage and transportation (9). In this study, we compared a Gen-Probe DNA probe assay (GP) with culture for the detection of *N. gonorrhoeae* in a population of patients visiting the outpatient clinic of the Sexually Transmitted Diseases Department of the Westeinde Hospital. The study population had infection prevalence rates of 14.9 and 7.7% for men and women, respectively. The overall positive rate was 8.7%. Sensitivity of the DNA probe assay for the total population was 97.1%. Specificity of the assay for this group of patients was 99.1%. The predictive positive and negative values were 90.6 and 99.8%, respectively. These are comparable with the values previously reported by Panke et al. (10). The relatively high prevalence rate of our patient population (8.7%) affected the sensitivity to a lesser extent than reported in the study by Panke et al.

Of the 1,750 total specimens tested, there were 14 false positives with the probe assay. A total of 12 of the samples had sample RLU/cutoff RLU ratios that were higher than 2.0, strongly suggesting true gonorrheal infections. Five samples of the urethral discharges of six male patients were stained with methylene blue. Diplococci were visible in the stained discharges from all five of the patients tested. All 13 patients exhibited one or more characteristics that put them at high risk for sexually transmitted diseases. These included previous gonorrheal infections, promiscuity, and/or engaging in prostitution. Two of the patients were at high risk for sexually transmitted diseases even though they were asymptomatic for gonorrheal infections. The probe assay sample RLU/cutoff RLU ratios for their specimens were 1.3 and 1.4 (patients no. 8 and 12, respectively) (Table 4). In the case of patient no. 12, we could not find further evidence for gonococcal disease; in patient no. 8, the urethral specimen RLU ratio was 41.1 and could be seen as positive. Technical problems, including inoculation of specimens onto cold media (in four cases, it appeared that the nurse had taken the solid media out of the refrigerator minutes before inoculation) and overgrowth by other bacteria, may have contributed to apparent probe false positives. Taking everything into consideration, the additional evidence presented for these 14 specimens suggests that they may be culture false negatives and not probe false positives. These data strongly suggest that the DNA probe assay may be more sensitive

than culture for detecting gonorrheal infection. The four samples yielding a positive result in culture and a negative result in the DNA probe assay could be explained by swab-to-swab variability. Because the DNA probe assay swab was taken consistently as the last swab, adverse patient reactions may have resulted in inadequate probe samples.

In the study by Panke et al. (10), 22 of 3,048 specimens tested in the Gen-Probe assay had sample RLU/cutoff RLU ratios between 0.7 and <2.0. On repeated testing of the 22 specimens, 12 were negative for *N. gonorrhoeae* and had ratios between 0.7 and <1.0, whereas 10 were positive and had ratios between 1.0 and <2.0. Similarly, in our study, we found 11 samples with ratios between 0.7 and <2.0. Of these, five samples had probe ratios between 0.7 and <1.0 and were negative in culture and probe assay, whereas six samples had probe ratios equal to or higher than 1.0 and less than 2.0 and were positive by culture and/or probe assay. These samples represented 0.4% of all negative tests and 3.9% of all positive tests. All 11 samples were repeatedly tested in our study. We feel that this region around the cutoff should be considered suspect and that new patient samples should be obtained.

No study evaluating the diagnostic value of the GP for specimens from nongenital sites has been published. Nongenital samples were taken from the pharynges ($n = 137$) and the rectums ($n = 46$) of 183 patients. In this study, we found eight patients (Table 3) with positive probe results in pharyngeal or rectal specimens; one specimen was negative by culture. The prevalence of *N. gonorrhoeae* on nongenital sites was 4.3%, and sensitivity and specificity values were 100 and 99.4%, respectively. Positive and negative predictive values, respectively, were 87.5 and 100% (Table 2). Patient no. 7 (pharynx) (Table 4) was culture negative, but on the basis of the clinical signs of STD and tonsilitis, his sexual behavior history, and the GP-positive RLU ratio of 3.8, we considered this patient positive for gonorrheal infection. Further study to obtain a larger series of specimens from nongenital sites to evaluate will be necessary.

rRNA as a control marker after treatment has shown its usefulness, although the number of patients was small. No false-positive reactions were seen after adequate patient treatment. Two patients with renewed contacts who were

positive in the probe assay within 10 days of treatment were considered reinfections.

One disadvantage of the DNA probe test is its inability to provide information on β-lactamase production by *N. gonorrhoeae*. In our region, with an *N. gonorrhoeae*-positive population with β-lactamase production of about 50%, treatment with ceftriaxone is a recommended guideline. Because of the earlier findings from surveillance of β-lactamase-producing strains and of the emergence of resistance to (for instance) ceftriaxone, epidemiological tracking of antibiotic resistance may be important in the future. Patient samples collected during certain periods should be tested in DNA probe assay and culture.

The advantage of using this DNA probe technique is that simultaneous testing for *C. trachomatis* of the same specimen is possible. We tested 1,119 of the patients in this study for the presence of *C. trachomatis*. We detected 101 probe-positive *Chlamydia* samples in specimens collected from 87 patients (data not shown). A total of 13 of the *Chlamydia*-positive patients had concomitant gonorrheal infections. On the basis of data from an earlier evaluation in our laboratory (unpublished data) and of data published by Kluytmans et al. (5), the Gen-Probe PACE 2 assay to detect *C. trachomatis* has been fully accepted in our laboratory setting.

In conclusion, the DNA probe test is useful as a suitable screening and diagnostic test for gonorrheal infection in men and women. An advantage of using this DNA probe technique is the fact that the patient specimen can be tested simultaneously for *C. trachomatis*. The DNA probe assay might also be suitable to use for specimens from nongenital sites. By using rRNA as a control marker after treatment, it may be possible to use the Gen-Probe assay for testing whether patients have been cured. Because the numbers of patients tested were low, further studies are required to determine whether the preceding two statements will hold true.

ACKNOWLEDGMENTS

REFERENCES

1. **Aardoom, H. A., D. D. Hoop, C. O. A. Iserief, M. F. Michel, and E. Stolz.** 1982. Detection of *Neisseria gonorrhoeae* antigen by a solid-phase enzyme immunoassay. Br. J. Vener. Dis. **58:**359–362.
2. **Anonymous.** Annual report infectious diseases. 1991. NTVG **135(9):**400.
3. **Burns, M., P. H. Rossi, D. W. Cox, T. Edwards, M. Kramer, and S. J. Kraus.** 1983. A preliminary evaluation of the Gonozyme test. Sex. Transm. Dis. **10:**180–183.
4. **Granato, P. A., and M. R. Franz.** 1989. Evaluation of a prototype DNA probe test for the noncultural diagnosis of gonorrhea. J. Clin. Microbiol. **27:**632–635.
5. **Kluytmans, J. A. J. W., H. G. M. Niesters, J. W. Mouton, W. G. V. Quint, J. A. J. Ijpelaar, J. H. Van Rijsoort-Vos, L. Habbema, E. Stolz, M. F. Michel, and J. H. T. Wagenvoort.** 1991. Performance of a nonisotopic DNA probe for detection of *Chlamydia trachomatis* in urogenital specimens. J. Clin. Microbiol. **29:**2685–2689.
6. **LeBar, W., B. Herschman, C. Jemal, and J. Pierzchala.** 1989. Comparison of DNA probe, monoclonal antibody enzyme immunoassay, and cell culture for the detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **27:**826–828.
7. **Lefebvre, J., H. Laperrière, H. Rousseau, and R. Massé.** 1988. Comparison of three techniques for detection of *Chlamydia trachomatis* in endocervical specimens from asymptomatic women. J. Clin. Microbiol. **26:**726–731.
8. **Lewis, J. S., D. Kranig-Brown, and D. A. Trainor.** 1990. DNA probe confirmatory test for *Neisseria gonorrhoeae*. J. Clin. Microbiol. **28:**2349–2350.
9. **Limberger, R. J., R. Biega, A. Evancoe, L. McCarthy, L. Slivienski, 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.
10. **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 detection of *Neisseria gonorrhoeae* in endocervical specimens. J. Clin. Microbiol. **29:**883–888.
11. **Roefaro-Franz, M., and P. A. Granato.** 1990. Use of the Gen-Probe PACE System for the detection of *Neisseria gonorrhoeae* in urogenital samples. Diagn. Microbiol. Infect. Dis. **13:**217–221.
12. **Stamm, W. E.** 1985. Diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections using antigen detection methods. Diagn. Microbiol. Infect. Dis. **4:**935–995.
13. **Stamm, W. E., B. Cole, C. Fennell, P. Bonin, A. S. Armstrong, J. E. Herrmann, and K. K. Holmes.** 1984. Antigen detection for the diagnosis of gonorrhea. J. Clin. Microbiol. **19:**399–403.
14. **Stapinski, A., Z. Seliborska, and B. Matus.** 1989. Usefulness of the gonozyme (Abbott) test in the diagnosis of gonorrhea in prostitutes. Przegl. Dermatol. **76:**58–63.
15. **Totten, P. A., K. K. Holmes, H. H. Handsfield, J. S. Knapp, P. L. Perine, and S. Falkow.** 1983. DNA hybridization technique for the detection of *Neisseria gonorrhoeae* in men with urethritis. J. Infect. Dis. **148:**462–471.