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Comparison of Gen-Probe DNA Probe Test and Culture for the Detection of *Neisseria gonorrhoeae* in Endocervical Specimens

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A 2-h nonisotopic DNA probe assay for the direct detection of *Neisseria gonorrhoeae* in urogenital specimens has recently been modified (PACE 2; Gen-Probe, San Diego, Calif.). The new assay format was developed to increase the sensitivity of the assay and simplify procedural steps. In this study, the new DNA probe test was compared with a culture reference method for the detection of *N. gonorrhoeae* in endocervical specimens. The results of the DNA probe test were expressed as a ratio of relative light units (RLU) of the specimen/RLU of the cutoff recommended by the manufacturer. All patient samples with sample RLU/cutoff RLU ratios less than 0.7 were interpreted as negative, and ratios greater than 2.0 were interpreted as positive for gonorrhea. Samples with sample RLU/cutoff RLU ratios between 0.7 and 2.0 were repeated until two or more consistent negative or positive ratios were obtained. A total of 469 specimens were tested with an overall disease prevalence of 6.1%. Of the 469 patients tested, 5 specimens (1.0%) fell in this borderline region and were retested. If the manufacturer's recommended cutoff value had been used, the original DNA probe results would have resulted in two false-positives. Our data were analyzed for both symptomatic (prevalence, 11.7%) and asymptomatic (prevalence, 2%) women. The study indicated that with our modification of the manufacturer's endpoint interpretation, the DNA probe test was essentially equivalent to the culture method in terms of sensitivity, specificity, and positive and negative predictive values in both symptomatic and asymptomatic patient populations. The new DNA probe test can serve as a suitable screening and diagnostic test for the diagnosis of gonorrheal genital infections in women. Additionally, it offers the advantages of rapid turnaround time and ease of use and allows simultaneous testing for *Chlamydia trachomatis* on the same specimen.

Gonorrhea remains one of the most important sexually transmitted diseases worldwide, with a reported 720,000 cases in the United States in 1988 (1). Gynecologic infections can range from asymptomatic to mildly symptomatic to severe, with long-term serious sequelae including infertility, chronic pelvic pain, and ectopic pregnancy. It has also been implicated in obstetric complications, neonatal pneumonia, and conjunctivitis. The control of epidemic infections and their sequelae remains dependent on correct and timely diagnosis.

The time-honored standard for diagnosis of genital gonorrhea has been Gram stain and culture, using self-generating carbon dioxide transport devices containing selective growth medium (5, 7). Conventional culture procedures are dependent on the presence of viable bacteria for microbial isolation. Nonculture alternatives for the laboratory detection of *Neisseria gonorrhoeae* in urogenital specimens include enzyme immunoassay (11) and nucleic acid hybridization (4, 12).

In this report, we describe the diagnostic evaluation of the Gen-Probe DNA probe assay (PACE 2; Gen-Probe, Inc., San Diego, Calif.) for the detection of *N. gonorrhoeae* directly in urogenital specimens. This 2-h nonisotopic DNA probe assay has recently been modified. The new assay format was developed to increase the sensitivity of the assay and to reduce the number of procedural steps.

The objectives of this study were the following: (i) to compare the new DNA probe assay format to a culture reference method for the detection of *N. gonorrhoeae* in endocervical swab specimens from asymptomatic and symptomatic women and (ii) to determine whether using a discrete manufacturer-recommended cutoff value is sufficient for unequivocal, clear-cut results for all patient specimens.

(Part of this work was presented previously [6].)

MATERIALS AND METHODS

Patient population. Two groups of patients were included in this study. Group I patients were asymptomatic pregnant women who came for prenatal care. The clinic charts of these patients were reviewed to assure that the patients had nonspecific or no symptoms of gonorrheal infection and were not on antibiotics at the time of specimen collection. Group II patients consisted of symptomatic women who came for gynecologic care. All patients were seen at Good Samaritan Hospital Outpatient Clinic or Group Health Associates, Cincinnati, Ohio, between September 1989 and June 1990.

Specimen collection. The exocervix was first cleansed with a Dacron swab. Subsequently, two endocervical samples were collected, one for the DNA probe assay and one for the culture method. The order of sample collection was random. Specimens collected for the DNA probe test were placed in Gen-Probe transport tubes containing 1.0 ml of specimen preservative and glass beads. Samples were stored at room temperature for up to 1 week prior to being tested. All study samples were saved at −70°C for further testing if discrepancies arose. For culture, swabs were inoculated onto modified Thayer-Martin plates (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.), warmed to room

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temperature, and transported to the laboratory in carbon dioxide Bio-Bags (Marion Scientific, St. Louis, Mo.).

DNA probe assay. The Gen-Probe PACE 2 system chemiluminescent labeled DNA probe test was used in the study. This system uses an acridinium ester-labeled single-stranded DNA probe that is complementary to rRNA of *N. gonorrhoeae*.

All reagents and samples were brought to room temperature before use. Each sample was vortexed for 10 to 15 s, and swabs were expressed of liquid on the side of the tube and then discarded. The specimen was vortexed, and then 100 μl of sample was aliquoted into a tube. Reconstituted probe reagent (100 μl) was then pipetted into 100 μl of the aliquoted sample. The tubes were covered with a sealing card, and the tube rack was shaken three to five times to mix. The mixture was incubated at 60 ± 1°C in a water bath for 1 h. One milliliter of separation solution was added to each tube and then incubated at 60 ± 1°C for 10 min. The separation rack was placed onto a magnetic base at room temperature for 5 min.

The supernatant was decanted, and the tubes were blotted on absorbent paper. Each tube was filled to the rim with wash solution. The tubes remained on the separation rack for 20 min at room temperature. The supernatant was then decanted but not blotted. The tube rack was shaken to resuspend the pellets. For each set of specimen runs, three negative controls and one positive control were simultaneously processed.

The tubes were read on the Leader I luminometer (Gen-Probe, Inc.) with the gonorrhea protocol selected. The results of the test were calculated based on the difference between the response relative light units (RLU) of the specimen and the mean RLU of the negative reference.

Culture. Specimens collected with Culturette II Dacron swabs (Marion Scientific) were inoculated onto modified Thayer-Martin plates (BBL, Becton Dickinson Microbiology Systems) warmed to room temperature. The samples were transported to the laboratory in carbon dioxide Bio-Bags (Marion Scientific). Cultures were incubated at 35°C for up to 72 h and examined daily for growth. Isolates were identified as *N. gonorrhoeae* by standard procedures on the basis of growth and colony characteristics, Gram stain morphotype, oxidase reaction, and carbohydrate utilization using Quadferm (API Analytab Products, Plainview, N.Y.).

Discrepant results. A DNA probe test was performed on colonies from cultures of patient specimens that were positive for *N. gonorrhoeae* by culture but negative for *N. gonorrhoeae* by DNA probe test. In addition, all those specimens that were positive by DNA probe assay and negative by culture were submitted in a blind manner to another laboratory (Gen-Probe) for the probe competition assay. Half-volume reactions (50 μl of sample and 50 μl of probe) were used for all probe competition assays. Three separate assays were set up for each specimen: (i) sample and labeled *N. gonorrhoeae* probe; (ii) sample, labeled *N. gonorrhoeae* probe, and 100× unlabeled homologous probe (*N. gonorrhoeae*); (iii) sample, labeled *N. gonorrhoeae* probe, and 100× unlabeled heterogeneous probe (*Mycoplasma pneumoniae*). Results for the probe competition assay were interpreted as follows: a 90% reduction in RLU difference (competition) in tube 2 versus tubes 1 and 3 indicated that a specific hybridization reaction took place and that *N. gonorrhoeae* nucleic acids were present in the sample. Less than a 90% reduction in RLU difference (no competition) indicated that a specific hybridization reaction did not take place and that *N. gonorrhoeae* nucleic acids were not present in the sample. True positive samples were identified in this study as those specimens that were positive by culture or positive by two nonculture tests (i.e., DNA probe test and probe competition assay) if culture was negative.

TABLE 1. Interassay precision data

Sample	No. of DNA tests run	Sample RLU/cutoff RLU ratio		
		Mean	SD	Coefficient of variation (%)
Negative control[a]	26	0.14	0.02	14.3
Positive control	25	4.21	0.64	15.2
Patient no.				
364	6	0.69	0.20	29.0
228	6	0.90	0.15	16.7
031	5	1.90	0.35	18.4
099	5	3.00	0.60	20.0
188	5	5.49	1.60	29.0
322	4	8.30	2.10	25.0
275	5	27.90	7.00	25.0
120	4	62.00	15.90	26.0

[a] Two negative controls had values greater than 3 standard deviations from the mean (negative control 1 had a ratio of 0.73; negative control 2 had a ratio of 0.32), and they were not used to produce the above results.

Cutoff range for the DNA probe assay. The results of the DNA probe test are calculated based on the difference between the response in RLUs of the specimen and the mean of three negative reference values. According to the manufacturer, the specimen should be considered as positive for *N. gonorrhoeae* if the difference between the specimen response and the mean of the negative reference is greater or equal to 300 RLU. One of the objectives of the study was to determine whether using such a discrete manufacturer-recommended cutoff value is sufficient for unequivocal, clear-cut results for all patient samples. To address this question, we obtained interassay precision data for samples at various levels from the cutoff value, examined the distribution of RLU values for all patient samples in relation to the cutoff value, and correlated the DNA probe test results with culture results and with clinical data. We expressed all patient RLU values as a ratio of RLU of patient sample/RLU of the recommended cutoff. In this manner, all ratios equal to or greater than 1 represented patient specimens that were positive for *N. gonorrhoeae*. All ratios less than one represented patient specimens that were negative for *N. gonorrhoeae*. Additionally, the further a given ratio deviated from the value of 1.0, the more clear-cut negative or positive results were obtained.

RESULTS

Samples that tested negative for *N. gonorrhoeae* by the DNA probe assay. Table 1 shows between-run precision data for the DNA probe assay. This interassay precision data was calculated by assaying the same sample in several consecutive runs. A negative control sample, a positive control sample, and several patient samples with various sample RLU/cutoff RLU ratios were evaluated. The coefficients of variation for the negative and positive control samples were approximately 15% and the coefficients of variation for the patient samples were as high as 29% (Table 1). With such interassay variation, occasional patient samples that are truly positive for *N. gonorrhoeae* (ratio of ≥1.0) could on a single run have a ratio as low as 0.7. In this study, we determined what percentage of negative patient samples fell

TABLE 2. Distribution of RLU values in patients who tested negative for gonorrhea by the DNA probe assay

Range of sample RLU/cutoff RLU ratios	No. of patients (%)	
	Study patients[a] (DNA assay and culture)	Additional patients[b] (DNA assay only)
1.0 > ratio ≥ 0.9	0	4 (0.2)
0.9 > ratio ≥ 0.8	0	1 (0.04)
0.8 > ratio ≥ 0.7	1 (0.2)	6 (0.25)
0.7 > ratio ≥ 0.6	0	7 (0.3)
0.6 > ratio ≥ 0.5	0	15 (0.6)
0.5 > ratio ≥ 0.4	3 (0.7)	28 (1.1)
0.4 > ratio ≥ 0.3	16 (3.6)	54 (2.2)
0.3 > ratio ≥ 0.2	52 (11.7)	247 (10.1)
0.2 > ratio ≥ 0.1	355 (80.0)	2,005 (81.9)
0.1 > ratio ≥ 0.0	17 (3.8)	79 (3.3)
Total	444 (100)	2,446 (100)

[a] 469 study patients were evaluated for the presence of gonorrhea by DNA probe assay and by culture. When the manufacturer-recommended cutoff was used, 444 (94.7%) of the study patients were negative for gonorrhea by the DNA probe assay.

[b] 2,578 patients were evaluated for the presence of gonorrhea by the DNA probe assay alone. When the manufacturer-recommended cutoff was used, 2,446 (94.9%) of the patients were negative for gonorrhea by the DNA probe assay.

TABLE 3. Distribution of RLU values in patients who tested positive for gonorrhea by the DNA probe assay

Range of sample RLU/cutoff RLU ratios	No. of patients (%)	
	Study patients[a] (DNA assay and culture)	Additional patients[b] (DNA assay only)
ratio ≥ 10.0	17 (65.4)	104 (78.8)
10.0 > ratio ≥ 7.0	0 (0.0)	6 (4.5)
7.0 > ratio ≥ 6.0	0 (0.0)	4 (3.0)
6.0 > ratio ≥ 5.0	1 (3.8)	5 (3.8)
5.0 > ratio ≥ 4.0	1 (3.8)	3 (2.3)
4.0 > ratio ≥ 3.0	1 (3.8)	1 (0.7)
3.0 > ratio ≥ 2.0	2 (7.7)	3 (2.3)
2.0 > ratio ≥ 1.5	2 (7.7)	3 (2.3)
1.5 > ratio ≥ 1.0	2 (7.7)	3 (2.3)
Total	26 (100)	132 (100)

[a] 469 study patients were evaluated for the presence of gonorrhea by DNA probe assay and by culture. When the manufacturer-recommended cutoff was used, 26 (5.5%) of the study patients were positive for gonorrhea by the DNA probe assay.

[b] 2,578 patients were evaluated for the presence of gonorrhea by the DNA probe assay alone. When the manufacturer-recommended cutoff was used, 132 (5.1%) of the patients were positive for gonorrhea by the DNA probe assay.

into this borderline region (0.7 ≥ ratio ≤ 1.0) and whether repetitive DNA probe testing of these samples allowed for consistent and clinically relevant results.

Table 2 shows the distribution of sample RLU/cutoff RLU ratios for all those samples that tested negative for *N. gonorrhoeae* by the DNA probe assay. Using the manufacturer-recommended cutoff, 444 of 469 patients in this study were negative for gonorrhea. More than 99% of these negative samples were at least 50% away from the cutoff value (ratio of ≤0.5) (Table 2). Only one study patient had a sample RLU/cutoff RLU ratio greater than 0.5 (i.e., 0.7). This patient was negative for *N. gonorrhoeae* by culture. Repetitive testing by DNA probe assay was performed on this patient's sample in five separate assays. The following ratios were obtained: 0.70, 0.93, 0.86, 0.91, and 1.11 ($\bar{x}$ = 0.90 ± 0.1). Additionally, randomly selected samples from patients with ratios of ≤0.5 were assayed several times and these remained negative on repetitive testing (data not shown).

Since only 1 study patient had a sample RLU/cutoff RLU ratio between 0.5 and 1.0, an additional 2,578 patient samples were analyzed by DNA probe assay during October 1989 and June 1990. The sample/cutoff ratios for all these patients were determined (Table 2). These additional samples were evaluated for the presence of *N. gonorrhoeae* by DNA probe assay only, and no matching cultures were performed. Of 2,578 samples analyzed, 2,446 (94.6%) specimens were negative for *N. gonorrhoeae*. As with the study patients, only a small number of patients who tested negative for *N. gonorrhoeae* (1.4%) had sample RLU/cutoff RLU ratios between 0.5 and 1.0 (Table 2). Of these, six patients had ratios between 0.7 and 0.8. Two of these six patients had samples available for repetitive DNA probe testing. Both samples remained negative on repetitive DNA probe testing. Of the 2,446 negative specimens, one had a sample RLU/cutoff RLU ratio between 0.8 and 0.9 (Table 2). The quantity of this one sample was insufficient for repetitive testing. Four patients had 1.0 > ratio ≥ 0.9 on primary testing. On repetitive testing, two of these four patients remained negative for *N. gonorrhoeae* (i.e., patient 390 ratios = 0.98, 0.62, and 0.50 and patient 344 ratios = 0.9, 0.72, and 0.60). The other two of the four patients were positive for *N. gonorrhoeae* (i.e., patient 391 ratios = 0.9, 1.47, and 1.28 and patient 367 ratios = 0.9, 1.3, and 1.2).

Samples that tested positive for *N. gonorrhoeae* by the DNA probe assay. Table 3 shows sample RLU/cutoff RLU ratios in all those patient specimens that tested positive for *N. gonorrhoeae* by the DNA probe assay. The ratios are shown for all study patients (patients who had a DNA probe assay and a matching culture) and for 2,578 patient samples evaluated only by the DNA probe assay. Ninety percent of those patients who tested positive for gonorrhea by the DNA probe assay had sample RLU/cutoff RLU ratios of ≥2.0. Randomly selected patients with sample RLU/cutoff RLU ratios of ≥2.0 were repetitively tested for *N. gonorrhoeae* by the DNA probe assay and all of these remained positive on repetitive testing (data not shown). The two study patients who had 3.0 > ratio ≥ 2.0 (Table 3) were also positive for *N. gonorrhoeae* by culture.

Four study patients (0.8%) were DNA probe assay positive for *N. gonorrhoeae*, with ratios less than 2.0. Two of these four patients became negative for *N. gonorrhoeae* on repetitive testing (i.e., patient 345 ratios = 1.07, 0.44, and 0.42; patient 378 ratios = 1.9, 0.9, and 0.88). Both of these patients were negative for *N. gonorrhoeae* by culture and by the probe competition assay. The other two patients with sample/cutoff ratios of <2.0 remained positive for *N. gonorrhoeae* on repetitive testing (i.e., patient 311 ratios = 1.37 and 1.40; patient 345 ratios = 1.77 and 1.89). Patient 311 was also positive for *N. gonorrhoeae* by culture. Patient 345 was positive for *N. gonorrhoeae* by the probe competition assay in addition to being positive by repetitive DNA probe testing.

From the 2,578 patients who had only the DNA probe test performed, three patients (0.1%) had 2.0 > ratio ≥ 1.5. One of these patients became negative for *N. gonorrhoeae* on repetitive DNA probe testing.

TABLE 4. Comparison of DNA probe test and culture for detection of *N. gonorrhoeae* in asymptomatic pregnant patients and symptomatic patients

DNA probe test result	No. of culture results					
	Asymptomatic patients		Symptomatic patients		Asymptomatic and symptomatic patients	
	+	−	+	−	+	−
+	5	1	17	1	22	2
−	0	284	3	158	3	442

Of 2,578 patients evaluated for the presence of *N. gonorrhoeae* by the DNA probe test, 3 (0.1%) had 1.5 > ratio ≥ 1.0. Two of these three patients became negative for *N. gonorrhoeae* on repetitive DNA probe testing (i.e., patient 388 ratios = 1.0, 0.77, and 0.92; patient 366 ratios = 1.03, 0.57, 0.5, and 0.4), and one remained positive (i.e., patient 324 ratios = 1.12 and 1.2).

Correlation of the DNA probe assay results with those of culture and clinical data. All patient samples with sample RLU/cutoff RLU ratios less than 0.7 and greater than 2.0 were tested by the DNA probe assay only once. These samples were diagnosed as negative or positive for *N. gonorrhoeae* according to the manufacturer's recommendations.

The patient samples with 2.0 > ratio ≥ 0.7 were assayed several times. Samples were considered to be negative for *N. gonorrhoeae* by the DNA probe assay if they had results consistently less than 1.0 (i.e., 0.7 ≤ ratio < 1.0) or a single ratio equal to or greater than one (i.e., 2.0 > ratio ≥ 1.0), with all other results less than 1.0 (i.e., 0.7 ≤ ratio < 1.0). Samples were considered to be positive for *N. gonorrhoeae* by the DNA probe assay if they had results consistently equal to or greater than 1.0 (i.e., 1.0 ≤ ratio < 2.0) or a single ratio less than one (i.e., 0.7 ≤ ratio < 1.0), with all other results equal to or greater than 1.0 (i.e., 1.0 ≤ ratio < 2.0).

In this study, true positive samples were those specimens that were positive by culture or positive by two nonculture tests (i.e., DNA probe test and probe competition assay) if culture was negative. The results obtained by DNA probe and culture for asymptomatic and symptomatic patients are shown in Tables 4 and 5.

Of 290 asymptomatic pregnant women, all 5 with culture-positive gonorrhea (2% prevalence) also yielded positive results in the DNA probe test (Table 4). However, one patient was DNA probe positive while culture was negative. This sample was positive for *N. gonorrhoeae* on repetitive DNA probe testing (i.e., ratios 1.77 and 1.89). Blind DNA competition test confirmed the presence of *N. gonorrhoeae* nucleic acid in the patient sample. The sample RLU/cutoff RLU ratios for all those asymptomatic patients who were positive for *N. gonorrhoeae* ranged from 1.77 to 64.52. The DNA assay demonstrated 99.6% correlation with culture. The DNA probe test had a sensitivity of 100%, specificity of 100%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 100%. The culture method had a sensitivity of 83.3%, specificity of 100%, PPV of 100%, and NPV of 99.6% (Table 5).

In the symptomatic population, the prevalence of gonorrhea was 11.7%. The DNA probe assay demonstrated 98% correlation with culture. Three patients were positive by culture and negative by DNA probe test (Table 4). All three of these patient specimens were repeatedly tested with the DNA probe assay. The DNA probe assay was consistently strongly negative for all three samples (sample RLU/cutoff RLU ratios: patient 340 ratios = 0.16 and 0.13; patient 325 ratios = 0.28 and 0.26; and patient 336 ratios = 0.14, 0.15, and 0.15). Probe competition assays were performed on all three patient samples. These probe competition assays verified that *N. gonorrhoeae* nucleic acid was absent in all three specimens. The DNA probe tests performed on colonies from cultures from these three patients were positive for *N. gonorrhoeae*.

TABLE 5. Sensitivity, specificity, and predictive values for DNA probe test and culture[a]

Patient group and test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Asymptomatic				
DNA probe test	100	100	100	100
Culture	83.3	100	100	99.6
Symptomatic				
DNA probe test	85.7	100	100	98
Culture	95.2	100	100	99
Asymptomatic and symptomatic				
DNA probe test	88.9	100	100	99
Culture	92.6	100	100	99

[a] True positive samples were those specimens positive by culture or two nonculture tests (i.e., DNA probe test and probe competition assay) if culture was negative.

One sample from a patient in the symptomatic patient population was negative for *N. gonorrhoeae* by culture and positive for *N. gonorrhoeae* by the DNA probe test. The DNA probe test was performed several times on this sample, and consistently positive results were obtained (i.e., ratios of 3.59, 3.10, and 3.18). Probe competition assay confirmed the presence of *N. gonorrhoeae* nucleic acid in this patient's sample.

In the symptomatic population, the DNA probe test had a sensitivity of 85.7%, specificity of 100%, PPV of 100%, and NPV of 98%. The culture method had a sensitivity of 95.2%, specificity of 100%, PPV of 100%, and NPV of 99% (Table 5).

Table 4 shows the data from the DNA probe test and culture when the results from the asymptomatic and symptomatic patients were combined. Of the total 469 patients tested, *N. gonorrhoeae* was detected in 25 (5.2%) specimens by culture and in 24 (5.2%) specimens by the DNA probe test. In this study, all *N. gonorrhoeae* isolated from culture were β-lactamase negative. Overall, the DNA probe test demonstrated 98.9% agreement with culture. Both the culture and the DNA probe test had a specificity of 100%, PPV of 100%, and NPV of 99%. The sensitivity of the DNA probe test was 88.9% and that of culture was 92.6% (Table 5). There was no difference in results obtained by DNA probe test and culture as determined by chi-square analysis.

DISCUSSION

Infection by *N. gonorrhoeae* remains an important sexually transmitted disease in sexually active adults. Culture on selective media currently is considered the "gold standard," although the sensitivity is reported to be only 85 to 95% (10). Culture requires organism viability and timely transport under stringent conditions to the laboratory for processing.

Recombinant DNA technology is providing an alternative identification method that offers rapid 2-h results, as well as ease of specimen storage and transportation. This study compared culture to DNA probe assay for the detection of *N. gonorrhoeae* in symptomatic and asymptomatic patient populations. Our study population had infection prevalence rates of 2.0 and 11.7% for asymptomatic and symptomatic patients, respectively. These are comparable with the prevalence rates previously reported (9).

In the state of Ohio, infection by *N. gonorrhoeae* is a reportable disease. By law, routine prenatal screening of all pregnant women is also required. One of the objectives of this study was to evaluate whether this DNA probe test can be used as a screening test for the detection of gonorrhea in low-risk populations with a prevalence rate of ≤2%. Such a screening test should have low numbers of false-positive results, since clinical implications associated with false-positive results lead to needless antimicrobial therapy as well as to social and psychological problems.

In our asymptomatic pregnant patient population, only one patient was found to be positive by the DNA probe assay and negative by culture. However, when comparing a nonculture test with a high sensitivity to culture tests with sensitivities of 85 to 95%, data must be interpreted cautiously. Positive nonculture results might be interpreted as false-positives rather than culture results being false-negatives. These positive nonculture results could be considered as true positive results if the presence of the organism was confirmed by another nonculture test. A test utilizing methodology that is different from the original nonculture test (i.e., an antigen test) would serve as an ideal confirmatory test. However, antigen testing cannot be performed on specimens collected in the Gen-Probe transport medium. We attempted to address this issue by submitting discrepant specimens mixed with other known positive and negative samples to another laboratory for DNA probe competition assays. In this study, we accepted the number of true positives to be the sum of specimens positive by culture and positive by a DNA probe assay, with confirmation of the presence of *N. gonorrhoeae* nucleic acid in the sample by probe competition assay. In our asymptomatic patient population, the one patient who was positive for *N. gonorrhoeae* by DNA probe and negative by culture was found to have *N. gonorrhoeae* nucleic acid present in the sample by probe competition. The DNA probe assay gave no false-positive or false-negative results in this patient population. These results indicate that the DNA probe test can be used as a screening test for the detection of *N. gonorrhoeae*.

We obtained similar results with our symptomatic patient population. One patient was DNA probe test positive and culture negative for *N. gonorrhoeae*. The probe competition assay verified that *N. gonorrhoeae* nucleic acid was present in this patient's sample. Additionally, three symptomatic study patients were negative for *N. gonorrhoeae* by the DNA probe assay and positive for *N. gonorrhoeae* by culture. DNA probe competition assay was negative for these samples, verifying that rRNA for *N. gonorrhoeae* was not present in the specimens submitted for the DNA probe assay. DNA probe assay performed on culture isolates of these three patients was positive, indicating the absence of a nonhybridizing rRNA for these *N. gonorrhoeae* isolates.

Overall, the culture method gave two false-negative results and the DNA probe assay gave three false-negative results, giving sensitivities of 85.7% for the DNA probe test and 95.2% for culture. In the asymptomatic patient population, the sensitivities were 100% for the DNA probe test and 83.3% for culture. These results do not necessarily indicate that the DNA probe test was more sensitive than culture nor that the probe test was more sensitive in asymptomatic patients than in symptomatic patients. The low number of *N. gonorrhoeae*-positive specimens in our patient population (e.g., a prevalence rate in asymptomatic patients of 2%) can greatly affect the sensitivity when just one sample is discrepant between the probe test and culture. Patient specimens for culture and for the DNA probe test were collected by separate swabs. In previous culture-versus-culture evaluations of chlamydial infections, discrepancy rates between 5 and 13% have been reported as a result of sample variation between multiple swabs (3, 8). These chlamydial studies, however, may not directly relate to sampling discrepancies for gonorrhea, since *Chlamydia trachomatis* is an intracellular parasite and specimen quality is critical. *N. gonorrhoeae* should be present in pus alone, allowing for less-stringent sampling technique requirements. However, the assumption that sampling error was a likely source of discrepant results is supported by blind DNA probe competition tests. These probe competition tests gave negative results for all samples negative by DNA probe tests but positive by culture, indicating that rRNA for *N. gonorrhoeae* was not present in the specimens submitted for the DNA probe assay. DNA probe tests performed on colonies from cultures of patient specimens that were positive by culture but negative by DNA probe test were positive. This indicated that samples submitted for culture contained detectable DNA sequences and further supported the assumption that sampling error was the likely source of discrepant results.

One problem we found with the DNA probe test was the discrete manufacturer-recommended cutoff of 300 RLU above the mean of the negative reference. Our results indicated that all values falling within sample RLU/cutoff RLU ratios of 0.7 to 2.0 should be repeated until unequivocal results are obtained. Of 3,047 patient samples evaluated by the DNA probe assay in this paper, 22 patients (0.7%) fell into this range. Of these, 12 patients who tested negative on the primary run (0.4% of all negative patients) and 10 patients who tested positive on the primary run (6% of all positive patients) needed to be repetitively tested. Failure to make this modification to the manufacturer's cutoff interpretation would have resulted in two false-positive results in our study patients. DNA probe tests on the remaining patient samples in this borderline region gave consistent negative or positive values upon reassay.

The cost-effectiveness of the DNA probe test is another factor that must be considered. Our study indicates that this DNA probe test must be repeated in borderline cases until unequivocal results are obtained. Such repetitive testing does not significantly increase the cost per test, because only 0.7% of all specimens tested fall into the borderline range. This test is not cost-effective compared with culture if the volume of testing is low and if it is the only probe test performed. However, the patient specimens collected for *N. gonorrhoeae* testing can be simultaneously tested for *C. trachomatis*. DNA probe testing can become cost-effective when one technologist can simultaneously perform multiple analyses on one sample utilizing the same methodology.

Disadvantages of the DNA probe test include lack of information on β-lactamase production and the inability of this test to detect *N. gonorrhoeae* in nongenital sites. Current Centers for Disease Control guidelines recommend that all adult cases of gonorrhea be treated with ceftriaxone and doxycycline (2). Use of this regimen will provide appro-

priate therapy even without β-lactamase information. However, when ceftriaxone resistance develops, the laboratory performing the DNA probe test will not have a colony on which to perform susceptibility testing. To date, DNA probe tests have been evaluated only for urogenital specimens in adults. Cultures should be performed for specimens from nongenital sites (throat, rectal, etc.) and for testing samples from children as may result from child abuse cases. Culture method problems include vancomycin susceptibility, unusual growth requirements, and storage and transport errors which make the organism nonviable.

Gonorrheal infections are responsible for significant gynecologic morbidity, and its sequelae have an impact long after the initial infection. Treatment is simple and effective, but diagnosis relies on accurate and timely laboratory diagnosis. Our study indicates that under our modification of the manufacturer's endpoint interpretation, this DNA probe assay serves as a suitable screening and diagnostic test for the diagnosis of gonorrheal genital infection in women. Additionally, it offers the advantages of rapid turnaround time and ease of use and allows simultaneous testing for *C. trachomatis* on one specimen.

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