

Evaluation of a Prototype DNA Probe Test for the Noncultural Diagnosis of Gonorrhea

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A prototype, nonisotopic, chemiluminescent DNA probe test called the Gen-Probe PACE (Probe Assay-Chemiluminescence Enhanced) system for *Neisseria gonorrhoeae* (Gen-Probe, San Diego, Calif.) was compared with conventional Martin-Lewis culture medium in JEMBEC plates for the laboratory diagnosis of gonorrhea. This 2-h noncultural assay is based upon the use of an acridinium ester-labeled DNA probe. The rRNA-directed DNA probe hybridizes with the target rRNA, and the hybridized probe is separated from the unhybridized probe through the use of magnetic microparticles. The esterified acridinium is hydrolyzed from the hybridized probe by the addition of an alkaline hydrogen peroxide solution, resulting in the production of visible light which is measured in a luminometer. The amount of light generated is directly proportional to the amount of gonococcal target rRNA present in the sample. A total of 407 clinical specimens (203 urethral and 204 endocervical) were collected from high-risk walk-in patients attending a sexually transmitted disease clinic. Separate patient specimens were collected for culture on Martin-Lewis medium in JEMBEC plates and for DNA probe assay. Statistical analysis of the overall comparative results showed that the DNA probe assay had a sensitivity, specificity, and positive and negative predictive values of 93, 99, 97, and 99%, respectively, in a patient population with a gonococcal disease prevalence of 21%. The results of this comparative study showed that the prototype chemiluminescent DNA probe assay is a rapid and reliable noncultural alternative for the laboratory diagnosis of gonorrhea.

Gonorrhea is a disease of worldwide distribution and is the most commonly reported bacterial infection in the United States. Even though almost 800,000 cases of gonorrhea were reported in this country in 1987, the overall incidence of this disease has not increased since 1975 (4). An important factor in controlling the incidence of this epidemic disease has been the availability and widespread use of reliable test systems for the laboratory diagnosis of gonorrhea.

The conventional approach to the laboratory diagnosis of gonorrhea is based upon the cultural recovery of *Neisseria gonorrhoeae* from clinical specimens by using self-generating carbon dioxide transport devices (16) containing specialized selective media (9, 12, 17). An alternative to the cultural diagnosis of gonorrhea is the use of noncultural methods, such as enzyme immunoassay and nucleic acid probes, for the direct detection of gonococci in urogenital samples. These noncultural methods offer several advantages over conventional cultural procedures because the diagnosis is not dependent upon the presence of viable microorganisms for microbial isolation and turnaround times can be significantly reduced. One commercially available enzyme immunoassay system for the direct detection of gonococci in clinical material, called Gonozyne (Abbott Laboratories, North Chicago, Ill.), has been reported to give good correlation with culture results (1, 3, 6, 11, 21, 26, 27).

Another noncultural alternative for the detection of gonococci in urogenital samples is nucleic acid hybridization with DNA probes. A prototype rRNA-directed DNA probe called the Gen-Probe PACE (Probe Assay-Chemiluminescence Enhanced) system for *Neisseria gonorrhoeae* (Gen-Probe, San Diego, Calif.) has been developed for the noncultural detection of gonococci in urogenital samples. The purpose of this

study is to comparatively evaluate the prototype nonisotopic DNA probe assay with a conventional cultural procedure in detecting the presence of *N. gonorrhoeae* in urogenital samples collected from a high-risk patient population.

MATERIALS AND METHODS

Medium. Martin-Lewis (ML) medium in JEMBEC plates (16) was used for the inoculation of all urogenital samples for the cultivation of *N. gonorrhoeae*. The medium was obtained commercially (Remel, Lenexa, Kans.) and was prepared according to the formulation of Martin and Lewis (17).

Patient population. The patient population consisted of high-risk walk-in patients attending the Sexually Transmitted Disease Clinic at the Onondaga County Department of Health in Syracuse, N.Y. Histories were obtained and physical examinations were performed on all patients by physicians and trained nurses. Patients who had documented gonococcal infections and returned to the clinic for the test-of-cure (TOC) cultures 7 days after treatment were also included in this study.

Specimen collection, processing, and culture. A total of 407 urethral and endocervical specimens were collected from the study population by physicians and trained nurses. For female patients, the excess mucus from the cervical os was removed with a sterile swab and then was discarded. A dual-swab (Culturette II; Marion Laboratories, Kansas City, Kans.) technique was used to collect the endocervical sample by placing both Dacron-tipped swabs into the endocervical canal and rotating for 30 s. One swab was used to inoculate ML medium, and the other swab was placed in the Gen-Probe specimen transport tube containing 1.0 ml of a specimen preservative and two glass beads. Each male urethral sample was collected by using two separate Dacron-tipped swabs. Each applicator was inserted separately 2 to 4

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cm into the urethra, using a rotating motion to facilitate insertion and adequate specimen sampling. The first swab was used for inoculation of ML medium, while the second swab was placed in the special Gen-Probe specimen transport tube with preservative and glass beads. If the patient had a purulent urethral discharge, a third urethral swab sample was collected to prepare a smear for Gram stain examination.

The urethral and endocervical swab samples that were collected for culture were rolled slowly over the surface of ML medium in a JEMBEC plate in a large Z pattern to maximize transfer of the inoculum to the agar surface. A carbon dioxide-generating tablet was placed in the well of the JEMBEC chamber, and the plate was sealed in an individual zip-lock environmental pouch and immediately placed in a 35°C incubator.

All JEMBEC plates were incubated for 72 h and examined daily for the appearance of microbial growth. Bacterial isolates were identified as *N. gonorrhoeae* on the basis of colonial morphology, Gram stain, oxidase reaction, and pattern of carbohydrate utilization in cystine-tryptophan agar medium. In addition, a saline suspension of each gonococcal isolate was prepared by placing at least five colonies in a Gen-Probe transport tube and storing at -70°C for possible testing by the Gen-Probe chemiluminescence assay.

Chemiluminescence assay. The prototype Gen-Probe PACE system for *N. gonorrhoeae* was used in accordance with the instructions of the manufacturer (Gen-Probe, San Diego, Calif.) All components for the test reagents were supplied by the manufacturer and were prepared according to the prototype insert directions. Positive and negative control samples were also provided in this prototype kit and were included in each sample run.

The Gen-Probe prototype assay consisted of four basic steps: (i) sample preparation; (ii) hybridization; (iii) separation of hybridized from unhybridized probe; and (iv) chemiluminescence measurement. Sample preparation consisted of vortexing each urogenital sample for 3 to 5 s, rimming the swab against the side of the tube to remove the excess liquid, and discarding the swab. After sample preparation, the specimens were either stored up to 1 month at -70°C or immediately assayed by batch testing. The hybridization step consisted of adding 100 µl of Probe Reagent to a tube containing 100 µl of clinical sample and incubating it in a 60°C water bath for 2 h. Separation of the hybridized probe from the unhybridized probe was achieved by adding 1 ml of Activator Reagent into each tube, incubating it in a water bath at 60°C for 10 min, and immediately adding 1 ml of Separation Reagent into each tube. The Separation Reagent consists of a suspension of magnetic particles that specifically bind only to hybridized probe. After a 5-min incubation in the water bath, the tubes were placed in a specially designed magnetic separation unit that collects the magnetic spheres at the sides and bottom of the tube, thereby allowing the supernatant fluid that contains the unhybridized probe to be discarded. After three washes, 300 µl of Elution Reagent was added to each tube containing the hybridized probe to release the hybridized probe from the magnetic particles. The supernatant fluid was transferred to a separate tube and assayed for the amount of hybridized probe present in the sample by the addition of Detection Reagent, which hydrolyzes esterified acridinium from the probe DNA. The amount of light emitted from this reaction (chemiluminescence) was measured quantitatively in the Leader I luminometer. Interpretations of the assay were based upon the ratio of the

TABLE 1. Comparative culture and DNA probe results for male urethral specimens

DNA probe result	No. of urethral culture results		Total
	Positive	Negative	
Positive	38 ^a	0	38
Negative	2	163 ^b	165
Total	40	163	203

^a Includes one urethral specimen from TOC patient.

^b Includes six urethral specimens from TOC patients.

response (in relative light units) of the specimen and the mean of the three negative control samples. When the relative light unit ratio was less than 4.0, the test results were interpreted as negative, and when the relative light unit ratio was 4.0 or greater, the test results were interpreted as positive. The Leader I calculates the results automatically and prints a hard-copy test result for each sample tested. This Gen-Probe chemiluminescence assay may be used to detect the presence of *N. gonorrhoeae* directly in urogenital samples or to confirm the identification of possible gonococcal isolates.

Criteria for gonococcal infection and statistical analysis. The interpretive criteria for determining whether a patient had gonococcal infection were the cultural recovery of *N. gonorrhoeae* from clinical material and the observed presence of polymorphonuclear neutrophils with gram-negative intracellular diplococci in Gram-stained urethral smears obtained from symptomatic males. All Gen-Probe test results were compared with those of culture or Gram-stained smear standards. The statistical parameters of sensitivity and specificity and the accuracy of positive and negative predictive values were calculated by standard methods (23).

RESULTS

A total of 407 urogenital specimens were evaluated in this study. Table 1 shows the comparative culture and probe assay results for 203 urethral samples. Based on cultural results, 40 males were diagnosed as having gonorrhea (disease prevalence of 19.7%). The DNA probe assay correlated well with culture, with no false-positive and only two false-negative test reactions. The Gram-stained smears from the two patients with these false-negative test reactions were both negative for the presence of intracellular gram-negative diplococci. Seven TOC male patients were included in this study group, and excellent test correlation was obtained with the cultural results. One TOC patient was positive by both culture and probe assay. This patient probably represented a treatment failure or reinfection.

Table 2 shows the comparative culture and DNA probe assay results for 204 endocervical specimens in a patient population with a disease prevalence of 22.5%. Overall, the DNA probe assay gave good correlation with culture. With culture as the standard of reference, seven discordant (four false-negative and three false-positive) test results were observed. All three of the false-positive test reactions gave borderline positive-negative results with the DNA probe assay and were interpreted as positive. Importantly, perfect correlation was obtained between culture and probe assay for the 18 TOC endocervical samples.

The comparative statistical analyses of culture and DNA probe assay for each specimen type and total specimens

TABLE 2. Comparative culture and DNA probe results from endocervical specimens

DNA probe result	No. of endocervical culture results		Total
	Positive	Negative	
Positive	42	3	45
Negative	4	155 ^a	159
Total	46	158	204

^a Includes 18 endocervical specimens from TOC patients.

tested are presented in Table 3. For male urethral specimens, the probe assay had a sensitivity of 95%, specificity of 100%, and positive and negative predictive values of 100 and 98.8%; for endocervical samples, the sensitivity, specificity, and positive and negative predictive values were 91.3, 98.1, 93.3, and 97.5%, respectively. The overall statistical parameters for all specimens indicate that the prototype probe assay compares very favorably with routine culture in identifying infected and noninfected patients.

For six gonococcal isolates recovered from patient specimens (two urethral and four endocervical), the paired clinical samples gave negative DNA probe results. These six isolates of *N. gonorrhoeae* were tested with the DNA probe assay to determine if they lacked the target rRNA specific for the DNA probe. All six isolates gave strong positive results with the DNA probe assay.

DISCUSSION

The conventional approach to the laboratory diagnosis of gonorrhoea is dependent upon the cultural recovery and subsequent identification of *N. gonorrhoeae* from clinical material or the detection of neutrophils with gram-negative intracellular diplococci in a Gram-stained smear of urethral exudate. Although examination by culture, smear, or both is generally regarded as the reference standard for establishing the diagnosis of gonococcal urogenital infection, these tests are not absolute because false-negative results can occur (10, 17). In addition, several days may elapse before the cultural diagnosis of gonorrhoea can be established.

The use of molecular technology, such as DNA probes, for the diagnosis of gonorrhoea overcomes many of the problems associated with culture while offering the potential for significantly reducing turnaround times. The use of DNA hybridization for detecting *N. gonorrhoeae* in urethral exudate was first reported by Totten et al. (28) in 1983. The probe was a ³²P-labeled cryptic plasmid of gonococcus that had the sensitivity to detect 100 colony-forming gonococci or about 0.1 pg of plasmid DNA. Comparative evaluation of this solid-phase hybridization assay with culture showed that it was 100% specific and detected 46 of 52 culture-positive samples. However, five of the six gonococcal culture iso-

lates that were recovered from probe-negative clinical samples were found not to possess the cryptic plasmid (22, 28). Thus, the radiolabeled hybridization test was 98% sensitive in detecting gonococci that possessed the multicopy target gene in clinical specimens. Attempts to develop a probe against a gene common to and unique for all gonococci have been successful (14, 18). However, since the specific gene is present only as a single copy in each organism, detection is difficult when low numbers of organisms are present in the clinical sample.

Despite the promise of these early results, the solid-phase hybridization assay has several distinct limitations. (i) The test cannot be reliably performed on cervical specimens, allegedly due to the low numbers of organisms that may be present in such samples (15, 28). (ii) False-negative probe results will occur in samples containing gonococci that do not possess the target cryptic plasmid (22, 28). (iii) The overall technology of the solid-phase assay is labor intensive, with 3- to 4-day turnaround times, and is not suitable for the clinical laboratory. (iv) The ³²P-labeled probe, with the associated problems of radioactive-waste disposal and laboratory safety, has a short shelf life.

The prototype nonisotopic probe assay evaluated in this study offers several advantages over the radiolabeled [³²P]DNA probe, originally described by Totten and co-workers (28). First, the prototype probe is based on a chemiluminescence assay that uses no radioactive compounds and has a considerably longer reagent shelf life. Second, the rRNA-directed DNA probe has an increased sensitivity of several orders of magnitude over the conventional DNA-directed DNA probe. Third, the prototype DNA assay is based upon liquid-phase hybridization, which is faster, more efficient, and more sensitive than the more conventional solid-phase hybridization technology with nitrocellulose filters (7, 13, 25). In addition, liquid-phase hybridization technology allows for the easy separation of hybridized from unhybridized probe through the use of magnetic microparticles.

An important advantage of the prototype DNA probe test over the alternative noncultural enzyme immunoassay is that the probe test can be reliably used on female TOC specimens. A problem with the enzyme immunoassay method for the detection of gonococci in clinical material was the reported high incidence of false-positive test results encountered with cervical TOC specimens (11). This high rate of false-positive test results was allegedly due to the persistence of gonococcal antigen in cervical secretions for an indefinite period of time after treatment. With the chemiluminescent probe assay, no false-positive test results were encountered with any of the TOC specimens evaluated in this study.

A total of nine discordant (three false-positive and six false-negative) DNA probe results were encountered in this study. The three false-positive test results occurred only with endocervical specimens when each of these samples gave repeated borderline test results. Either of two explanations could account for these alleged false-positive DNA probe results. First, even though culture was used as the reference standard for identifying patients with gonococcal infection in this study, it cannot be regarded as absolute because culture can give false-negative results (10, 17). In fact, approximately 10% of gonococci do not grow on ML medium due to the inhibiting effects of antibiotics in the growth medium (2, 5, 8, 19, 20, 24). Second, females with gonorrhoea may be infected with as few as 4.0×10^2 CFU of gonococci (15). Since the recovery of gonococci on ML

TABLE 3. Statistical analyses of DNA probe and culture

Specimen type (no. of specimens)	%			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Urethral (203)	95	100	100	98.8
Endocervical (204)	91.3	98.1	93.3	97.5
Both (407)	93	99	96.7	98.7

medium can be inoculum dependent (29), low numbers of gonococci in the clinical sample may be recovered by culture but could give a false-positive or borderline DNA probe result. Of the six false-negative DNA probe results, four were from endocervical samples and the remaining two were from urethral specimens. Since it was uncertain whether these false-negative results were attributable to the inability of the probe DNA to detect the target RNA in certain strains of gonococci, the gonococcal isolates recovered from the six discrepant probe specimens were tested directly with the DNA probe assay. All six isolates produced strongly reactive test results, suggesting that the six false-negative test results may have been attributable to the normal sampling error and variation that can occur during specimen procurement rather than to the inability of the probe to detect target RNA produced by certain gonococcal strains.

The results of this study showed that the chemiluminescent DNA probe assay compared favorably with culture in detecting the presence of *N. gonorrhoeae* in urogenital samples collected from high-risk patients. As such, this prototype assay provides a rapid and reliable noncultural alternative for the laboratory diagnosis of gonorrhea. Test results can be available within 2 h of specimen receipt, or specimens may be stored for a week at room temperature before batch runs are performed. In addition, the nonisotopic nature and extended shelf life of this chemiluminescent probe is another attractive feature of this technology, which offers the potential for widespread application in the microbiology laboratory.

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