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Direct DNA Probe Assay for *Neisseria gonorrhoeae* in Pharyngeal and Rectal Specimens

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The direct detection of gonococcal DNA in rectal and pharyngeal specimens was evaluated by using a DNA probe-based assay (Gen-Probe, Inc., San Diego, Calif.). Rectal (234) and pharyngeal (608) swab specimens were obtained from 249 men and 372 women attending sexually transmitted disease clinics in Las Vegas and Reno, Nevada. The prevalence of gonococcal infection by culture at the pharyngeal and rectal sites was 2.9% (16 of 548 specimens) in women and 2.7% (8 of 294 specimens) in men. No false-positive reactions were observed among the 234 rectal specimens tested. Two probe-positive, culture-negative specimens were detected among the 361 pharyngeal specimens obtained from women. Both of these samples were confirmed as *Neisseria gonorrhoeae* by a probe competition assay. The overall correlation of the DNA probe test with pharyngeal and rectal cultures was 99.4% (837 of 842 cultures), with a sensitivity of 87.5% (21 of 24 cultures) and specificity of 99.7% (816 of 818 cultures). The positive and negative predictive values of the DNA assay were 91.3 and 99.8%, respectively. The direct DNA probe assay provides an alternative to culture screening for rectal and/or pharyngeal gonococcal infections.

The laboratory diagnosis of gonococcal pharyngeal and rectal infections is usually achieved by the isolation of *Neisseria gonorrhoeae* on selective media, followed by confirmatory identification (2). Because these sites often harbor other bacterial species, which may be misidentified as *N. gonorrhoeae*, a variety of biochemical and immunological tests have been developed to aid in the culture confirmation of *N. gonorrhoeae* (3, 7).

An alternative nonculture system (PACE 2; Gen-Probe, Inc., San Diego, Calif.) in widespread use for detecting *N. gonorrhoeae* in cervical and urethral specimens has been reported to be accurate in studies in which parallel testing of duplicate samples was performed (5, 11, 13). This method detects the presence of gonococcal rRNA in patient specimens with an acridinium ester-labeled DNA probe coupled with a chemiluminescent detection system.

Studies involving the testing of specimens from sites other than the urethra and cervix for *N. gonorrhoeae* and using DNA probes have not been widely reported; Vlaspolder et al. (16) recently reported on a DNA probe study of nongenital specimens. We report here the results of an evaluation comparing mailed direct DNA probe assay specimen with on-site conventional culture specimens for detecting and identifying *N. gonorrhoeae* in pharyngeal and rectal specimens from men and women seen in sexually transmitted disease clinics.

Specimens were collected from 621 consecutive patients (249 men and 372 women) seen in sexually transmitted disease clinics in Las Vegas and Reno, Nevada, during the period March 1991 through December 1991. At both clinic sites, two swabs each were used (Gen-Probe Specimen Collection Kit) to collect the pharyngeal and rectal specimens from each patient; one swab was rolled onto a modified Thayer-Martin plate, and the other swab was placed in the PACE 2 Gen-Probe transport solution. Rectal specimens were obtained by inserting a swab 2 to 4 cm into the anal canal. Pharyngeal specimens were collected by rigorous swabbing of the posterior pharynx, including the tonsillar areas. The order of swab collection was rotated on a weekly basis to minimize the overall effect of specimen variation and collection. Plated specimens were cross-streaked and incubated for 48 h at 35°C in a humidified atmosphere supplemented with carbon dioxide. Isolates were identified by standard procedures, i.e., Gram staining, oxidase reaction, and colony morphology. Presumptively positive isolates were confirmed by monoclonal fluorescent-antibody tests (Bartels Diagnostics, Bellevue, Wash.). All nongonococcal isolates were identified by relevant biochemical tests and any additional bacteriologic procedures as necessary (12).

Specimens collected for DNA probe testing were transported by mail from the collection sites to Atlanta, Ga., and tested within 1 week. The results of the DNA probe test are calculated based on the difference between the response in relative light units (RLU) of the specimen and the mean RLU of three negative control values. If the specimen signal exceeded the mean of the negative references by 300 RLU (cutoff), the sample was considered positive. A probe competition confirmatory test was performed at the Centers for Disease Control, Atlanta, Ga., on specimens yielding dis-

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TABLE 1. Comparison of DNA probe versus culture to detect *N. gonorrhoeae* in pharyngeal samples from 361 women

Culture result	No. of samples with result					
	DNA probe[a]			Competition assay[b]		
	+	−	Total	+	−	Total
+	5	1	6	7	1	8
−	2	353	355	0	353	353
Total	7	354	361	7	354	361

[a] Sensitivity, 83.3%; specificity, 99.4%; positive predictive value, 71.4%; negative predictive value, 99.7%.
[b] Results after resolution of discrepant results by a DNA probe competition assay. Sensitivity, 87.5%; specificity, 100.0%; positive predictive value, 100%; negative predictive value, 99.7%.

TABLE 2. Comparison of DNA probe versus culture for the identification of *N. gonorrhoeae* in pharyngeal samples from 247 men

DNA probe result	No. of samples with culture result[a]		
	+	–	Total
+	5	0	5
–	2	240	242
Total	7	240	247

[a] Sensitivity, 71.4%; specificity, 100.0%; positive predictive value, 100.0%; negative predictive value, 99.1%.

crepant results, i.e., positive DNA probe and negative culture sample. The probe competition assay entails a repeat test using the original sample and another test using the original sample plus unlabeled homologous *N. gonorrhoeae* probe. If the sample is a true positive, the result is a reduction of RLUs greater than 80% due to the probe competition between the standard and unlabeled probes (4, 8).

The prevalence of pharyngeal gonococcal infections in these sexually transmitted disease clinic populations was 2.1% (13 of 608 specimens). Two DNA probe-positive, culture-negative specimens were detected among the 361 pharyngeal specimens from women (Table 1). When retested with the probe competition assay, both specimens were confirmed as *N. gonorrhoeae* positive; i.e., the probe competition assay verified the presence of gonococcal nucleic acid in both of these samples. In addition, both of these patients were contacts of men diagnosed as having gonococcal urethral infections, strongly indicating these two specimens were false-negative culture results. The sensitivity of the DNA probe test was 87.5% after resolution of these two samples. No probe-positive, culture-negative specimens were observed in the pharyngeal samples obtained from men (Table 2). However, the DNA probe test did not detect two samples that were confirmed culture positive, yielding a test sensitivity of 71.4% (5 of 7 specimens).

The prevalence of rectal gonococcal infections as determined by culture was 4.7% (11 of 234 specimens). The majority of positive cultures (10 of 11) occurred in women. The sensitivity and specificity of the DNA probe test in both men and women was 100% compared with those of gonococcal culture (Table 3). Results shown in Table 4 illustrate the wide range of RLU readings obtained between the mean values of the gonococcal isolates (men, 3,426 RLU; women, 13,423 RLU), and the nongonococcal isolates (men, 72 RLU; women, 56 RLU). Most of the gonococcal isolates gave readings that were 2 to 3 orders of magnitude greater than those of the nongonococcal isolates tested.

Pharyngeal gonorrhea is usually asymptomatic but can be a source for disseminated disease and may persist with or without treatment (1, 17). Because pharyngeal gonorrhea is often more difficult to eradicate than genital infection and because significant complications such as disseminated gonococcal infection can ensue, there is a need to identify patients with pharyngeal infection (6). Our results indicate that the specificity of the DNA probe assay is excellent and that the assay is at least as accurate as culture with the capability to obtain a definitive result in less than 4 h. The sensitivity of the probe was acceptable for women (87.5%) but low for men (71.4%). However, a relatively small number of positive samples were obtained in this study. Further studies with larger numbers of positive samples will be necessary to accurately assess probe performance.

TABLE 3. Comparison of DNA probe versus culture to detect *N. gonorrhoeae* in rectal samples from 234 men and women

DNA probe result	No. of samples with culture result[a]		
	+	–	Total
+	11	0	11
–	0	223	223
Total	11	223	234

[a] Sensitivity, 100.0%; specificity, 100.0%; positive predictive value, 100.0%; negative predictive value, 100.0%.

TABLE 4. Distribution of RLU values of pharyngeal *Neisseria* and *Moraxella* species isolated

Organism (no.)	RLU (range) [mean] in samples from:	
	Men	Women
N. gonorrhoeae (13)	610–12,328 [3,426]	611–61,012 [13,423]
N. menigitidis (13)	46–107 [72]	43–76 [56]
N. lactamica (3)	61	45–63 [54]
M. catarrhalis (1)	0	111

N. gonorrhoeae may be isolated from the anal canal in 35 to 50% of women with gonorrhea, and this is the sole site of infection in up to 5% of women (9, 13–15). Several studies have suggested that fewer than 10% of anorectal gonococcal infections are symptomatic (10). The DNA probe assay results were in complete agreement with culture results on all 234 rectal specimens tested.

Again, as with the pharyngeal specimens, the total number of positive rectal specimens was low. Although further studies with larger number of positive specimens are needed, the excellent performance of the probe assay presented here strongly indicates the utility of the assay for rectal specimens. The PACE 2 assay, although not currently approved for use with pharyngeal or rectal specimens, appears promising for the testing of these nongenital sites.

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