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Evaluation of the PACE 2 *Neisseria gonorrhoeae* Assay by Three Public Health Laboratories

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The Gen-Probe PACE 2 DNA probe assay for *Neisseria gonorrhoeae* was compared with conventional culture techniques in three Florida public health laboratories with 436 patients (271 females and 165 males). The prevalence rates based on culture were 19.9, 55.8, and 33.5% for females, for males, and overall, respectively. Twenty-seven probe-positive specimens gave negative culture results. Twenty of these specimens were resolved as true positives after retesting with a probe competition assay. The resolved sensitivity, specificity, positive predictive value, and negative predictive value were 99.4, 99.6, 99.4, and 99.6%, respectively.

Diagnosis of gonorrhea relies on the successful growth of *Neisseria gonorrhoeae* in culture to determine oxidase reaction, Gram stain reaction, and colony and cell morphology and to perform confirmation tests (1, 4). Technical problems, variability of quality assurance program maintenance in the sexually transmitted disease (STD) clinic setting, and problems in the transportation of cultures from clinic to laboratory frequently result in unsuccessful culture attempts (2, 6, 7).

Alternatively, the Gen-Probe PACE 2 assay for *N. gonorrhoeae* uses nucleic acid hybridization to identify gonococcal RNA directly from the PACE transport medium following inoculation with a urogenital specimen. Therefore, many problems associated with culture may be eliminated. The PACE 2 assay uses a chemiluminescent acridinium ester-labeled DNA probe that is specific for the rRNA of *N. gonorrhoeae*. After a urogenital swab specimen is placed the PACE 2 transport medium, the organisms are immediately lysed, thus releasing their rRNA. The target rRNA is subsequently hybridized to a labeled DNA probe, and following the removal of nonhybridized probe, the amount of hybridized probe is measured in a luminometer.

The PACE 2 system was compared with conventional culture methods in three public health laboratories. A total of 436 urogenital swab specimens (271 cervical and 165 urethral) were collected in STD clinics. To eliminate a specimen order collection bias, culture and PACE 2 swabs were alternated during the collection. Culture was performed on modified Thayer-Martin medium with a 48-h incubation period. Gram stains were performed on oxidase-positive cultures to determine Gram stain reaction and cellular morphology. Cultures yielding a gram-negative diplococcus with a "coffee bean" morphology were considered *N. gonorrhoeae*.

The swabs for the PACE 2 assay were placed in Gen-Probe transport tubes and then frozen at −70°C to facilitate batch testing. Specimens were subsequently thawed at 4°C, vortexed, and added to reaction tubes in 100-μl volumes. Each specimen was tested according to the manufacturer's instructions.

A total of 146 specimens were culture positive for *N. gonorrhoeae*. The PACE 2 assay detected all but one of these specimens, yielding an initial sensitivity of 99.3%. Twenty-seven specimens gave positive probe results and negative culture results, for an overall specificity of 90.7%. The probe-positive discrepant samples, along with eight control specimens (five positive and three negative), were further evaluated by blind testing at Gen-Probe by an unlabeled probe competition assay procedure (3, 5). Three assay tubes were set up for each specimen. Tube A was a repeat of the PACE 2 assay. Tube B contained a 100-fold excess concentration of unlabeled homologous probe in addition to the normal concentration of labeled probe. Tube C contained a 100-fold excess concentration of unlabeled heterologous probe in addition to the normal concentration of labeled probe. All assays were carried out in 50-μl half-volume reactions to conserve specimen (3, 5). The amount of probe hybridization was expressed in relative light units (RLUs) and was determined by using a luminometer. A 90.0% reduction of the net signal of tube B compared with the net signal of tube A was interpreted as a positive result for probe competition, indicating the presence of *N. gonorrhoeae* rRNA.

Results of the Gen-Probe PACE 2 and comparisons with conventional culture are summarized in Table 1.

Of the 165 male specimens, 92 were positive by culture for *N. gonorrhoeae*, yielding a prevalence for males of 55.8% in our study. All 92 were correctly detected by the PACE 2 assay. Thirteen additional specimens were also positive by the probe assay. The initial sensitivity and specificity for male specimens were 100.0 and 82.2%, respectively. Fifty-four endocervical specimens were culture positive for *N. gonorrhoeae*; the prevalence for females in our study was 19.9%. All but one was positive in the PACE 2 assay. Fourteen additional endocervical specimens were also positive by DNA probe. Initial sensitivity and specificity for female specimens were 98.1 and 93.5%, respectively.

Twenty-seven specimens (13 from men and 14 from women) were DNA probe positive and culture negative. Results obtained with these specimens are shown in Table 2. Eleven specimens from men were Gram stain positive in the STD clinic. Twelve of the discrepant samples from male gave high RLU signals both in initial testing and in tube A of the probe competition assay. Probe competition was in excess of 99% for these specimens. The remaining discrep-

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TABLE 1. Comparison of DNA probe test and culture for male and female patients

Result type and patient group	No. of:					%				
	Specimens	True positives	False negatives	False positives	True negatives	Sensitivity	Specificity	PPV[a]	NPV[b]	Agreement
Initial										
Males	165	92	0	13	60	100.0	82.2	87.6	100.0	92.1
Females	271	53	1	14	203	98.1	93.5	79.1	99.5	94.5
Combined	436	145	1	27	263	99.3	90.7	84.3	99.6	93.6
Resolved										
Males	165	105	0	0	60	100.0	100.0	100.0	100.0	100.0
Females	271	60	1	7	203	98.4	96.7	89.6	99.5	97.0
Combined	436	165	1	7	263	99.4	97.4	95.9	99.6	98.2

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

ant sample (FL35) gave an original signal of 1,044 RLUs and demonstrated 98.9% competition. All 13 of the male specimens were positive in the probe competition assay, confirming the presence of *N. gonorrhoeae* rRNA in the specimen. The resolved sensitivity and specificity for males were 100.0 and 100.0%, respectively.

Seven of the 14 probe-positive, culture-negative endocervical specimens were positive in the probe competition assay. The probe competition assay did not confirm the presence of *N. gonorrhoeae* in six endocervical specimens. Repeat PACE 2 analysis of these specimens using 50-μl volumes gave negative results. One of these specimens (FL22) was 4 RLUs below the negative cutoff for the half-volume competition reaction and showed 97.3% competition. An additional specimen (FL15) could be repeat tested only in tube A because of insufficient volume. The repeat result was a strong positive reaction at 8,629 RLUs. A third specimen (FL20) gave a low positive reaction at 373 RLUs

TABLE 2. Discrepant probe competition assay analyses of probe-positive, culture-negative specimens

Specimen	Patient sex	Probe competition assay					Initial testing	
		Net RLU in tube:			% Competition[a]	Result	RLU	Result
		A	B	C				
FL17	F	22,432	99	27,025	99.6	+	112,872	+
FL14	F	25,930	119	23,435	99.5	+	93,921	+
FL5	F	11,980	49	12,303	99.6	+	88,064	+
FL31	F	13,374	51	10,057	99.6	+	86,136	+
FL34	F	5,844	10	5,468	99.8	+	53,722	+
FL28	F	242	1	219	99.6	+	1,738	+
FL32	F	297	4	226	98.7	+	1,318	+
FL15	F	8,562	INS[b]	INS	NA[c]	NA	38,711	+
FL22	F	146	4	ND[d]	97.3	−	1,192	+
FL27	F	87	−6	99	NA	−	1,860	+
FL18	F	67	−7	19	NA	−	669	+
FL23	F	56	−6	65	NA	−	635	+
FL30	F	40	−7	19	NA	−	388	+
FL20	F	112	71	52	NA	−	373	+
FL9	M	286,479	1,975	319,763	99.3	+	833,895	+
FL11	M	172,625	900	148,400	99.5	+	536,249	+
FL2	M	155,070	637	165,101	99.6	+	518,671	+
FL13	M	150,736	692	150,369	99.5	+	510,095	+
FL12	M	140,989	692	147,212	99.5	+	494,701	+
FL1	M	105,170	464	93,422	99.6	+	376,127	+
FL24	M	62,460	358	54,019	99.4	+	248,754	+
FL33	M	50,022	209	55,954	99.6	+	236,674	+
FL6	M	38,666	125	47,915	99.7	+	165,047	+
FL7	M	57,112	239	65,762	99.6	+	164,064	+
FL4	M	35,660	196	45,666	99.5	+	139,445	+
FL8	M	28,610	232	30,065	99.2	+	125,785	+
FL35	M	277	3	270	98.9	+	1,044	+

[a] Percent reduction of signal in tube B compared with net signal in tube A.
[b] INS, insufficient volume.
[c] NA, not applicable.
[d] ND, not determined.

on initial testing but did not repeat positive or compete in the probe competition assay. The remaining four specimens from women that were not confirmed as containing *N. gonorrhoeae* were all very low positives, with original RLUs ranging from 388 to 1,860 in initial testing. These four specimens represent only 1% of the combined data. One of four women involved had cervicitis with a greenish discharge and was a possible gonorrhea contact. Another was pregnant and had a vaginal discharge. A third patient was referred to the STD clinic because of a vaginal discharge. In the probe competition assay, none of the four specimens tested positive in tube A. Although the tube B data suggested that competition had occurred in these specimens, the original PACE 2 results were judged to be in error because of the failure of the samples to repeat positive in tube A. Therefore, the specimens were resolved as true negatives.

Following resolution analysis, 20 of the 27 probe-positive, culture-negative specimens were shown to contain nucleic acid specific for *N. gonorrhoeae*. The combined resolved sensitivity for males and females after probe competition was 99.4%, whereas specificity increased to 99.6%. The positive and negative predictive values were 99.4 and 99.6%, respectively.

The results for samples obtained from females in this study are comparable to those in other reports on the PACE 2 assay for *N. gonorrhoeae* (3, 5). A key similarity between this study and that of Limberger et al. (3) was the transport of specimens to a centralized testing laboratory. In both cases, a significant number of probe-positive, culture-negative results which, after resolution, were determined to be culture failures were obtained. The success of culturing is dependent on many factors, such as proper storage of media; sterile, correct plate inoculation with the patient's swab; accurate monitoring of incubation temperatures; proper CO_2 conditions; and prompt culture incubation after plate inoculation. Many clinics have extensive quality assurance programs to monitor these factors. In comparison, PACE 2 swabs require no special handling and remain stable for 1 week at room temperature. Thus, it appears that under some circumstances of specimen transport, the PACE 2 system allows better specimen integrity, facilitating prolonged storage and higher detection rates. Furthermore, the ability to freeze specimens for testing at a later date allows retesting to be done, if necessary. Another advantage is the ability to test a single specimen for both *N. gonorrhoeae* and *Chlamydia trachomatis*; specimens are tested for chlamydia by using the PACE 2 assay system specific for *C. trachomatis*. Use of both assay systems on the same specimen allows the detection of either microorganism and enables one to determine coinfection rates.

The list price for a Thayer-Martin plate is $2.19 (Remel) compared with the list price for the PACE 2 assay for *N. gonorrhoeae*, which is $5.45 per test plus the cost of the swab at $2.23 each. Therefore, cost may be viewed as a disadvantage of the PACE 2 assay, since culturing can be performed at a considerably lower cost. However, one must take into consideration the advantage of the PACE 2 system and the disease prevalence in a given population in conjunction with cost when deciding how to test for *N. gonorrhoeae*. When specimen transport is going to present any type of problem, the ability of the PACE 2 system to maintain specimen integrity is a major advantage. Also, in populations with significant coinfection rates, the ability to test a single patient's sample for both *C. trachomatis* and *N. gonorrhoeae* is an advantage over conventional culture.

Unlike STD clinics, family planning and obstetrical clinics are not as likely to treat patients without definite laboratory confirmation of STD. In these family planning and obstetrical clinics, the additional cost for PACE 2 may also be warranted if the clinic quality assurance program or culture transportation presents problems.

The inability to determine antibiotic resistance of positive specimens with the PACE 2 system is a disadvantage. Although this inability is not yet a problem, because of the lack of reported resistance to ceftriaxone, the utility of the assay will need to be reassessed should resistance occur.

DNA probe technologies offer the potential to accurately detect gonococcal infections with assays that are easily performed on large numbers of specimens and that yield results in less than 3 h. Moreover, many of the problems associated with culture transportation, environmental factors, and the STD clinic quality assurance programs for gonococcal media and cultures are eliminated.

However, in order to ensure proper performance of the PACE 2 assays, test panels designed for internal laboratory use are available from the manufacturer. As well, proficiency panels to be used in laboratory quality assurance programs are currently available from outside sources. The proficiency assays are very straightforward and easy to perform, because they are prepackaged kits which are tested by the standard PACE 2 assay protocol.

REFERENCES

1. **Ehret, J. M., F. N. Judson, and J. W. Biddle.** 1984. Gonorrhea, p. 42–78. *In* B. B. Wentworth and F. N. Judson (ed.), Laboratory methods for the diagnosis of sexually transmitted diseases. American Public Health Association, Washington, D.C.
2. **Goodhart, M. E., J. Ogden, A. A. Zaidi, and S. J. Kraus.** 1982. Factors affecting the performance of smear and culture tests for the detection of *Neisseria gonorrhoeae*. Sex. Transm. Dis. **9:**63–69.
3. **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.
4. **Morella, J. M., W. M. Janda, and M. Bohnhoff.** 1985. *Neiserria* and *Branhamella*, p. 176–192. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. **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.
6. **Sng, E. H., V. C. Rajan, K. L. Yeo, and A. J. Goh.** 1982. The recovery of *Neisseria gonorrhoeae* from clinical specimens: effect of different temperatures, transport times, and media. Sex. Transm. Dis. **9:**74–78.
7. **Spence, M. R., D. S. Guzick, and L. R. Katta.** 1983. The isolation of *Neisseria gonorrhoeae*: a comparison of three culture transport systems. Sex. Transm. Dis. **10:**138–140.