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Enzyme Immunoassay for Diagnosis of Gonorrhea

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An enzyme immunoassay (EIA; Gonozyme [Abbott Laboratories]) for gonococcal antigen was assessed for the rapid diagnosis of gonorrhea. Patients attending two sexually transmitted disease clinics were tested by EIA and culture on Thayer-Martin medium. EIA was highly effective in detecting gonococcal infection among symptomatic men, with 70 of 75 (93.3%) culture-positive men having positive tests and no false-positive reactions. The performance of the test was not as good in detecting cervical gonorrhea; the best result obtained was a sensitivity of 87% (33 of 38) for EIA compared with culture. EIA false-positives occurred at a relatively low rate for women, with the test having a specificity of ca. 97%. The test clearly is capable of detecting gonococcal antigen in cervical and urethral specimens, but its role in routine diagnosis is not clear. Its performance seems equal to that of the Gram stain for men, but it seems to be less sensitive than culture for cervical gonorrhea—a drawback in high-risk populations. The low false-positive rate could be an important issue in screening low-prevalence populations.

Neisseria gonorrhoeae is an important human pathogen. Each year in the United States, several million diagnostic tests are performed for gonococcal infection. The diagnosis in symptomatic men is most often based on a Gram-stained smear of urethral discharge, but the Gram stain is not a particularly sensitive or specific test for diagnosis of asymptomatic carriers or infection of the lower genital tract of women (4). The recommended procedure for diagnosing the infection in women involves culture of a cervical swab specimen on Thayer-Martin (TM) or similar media. Cultural procedures are recognized to have sensitivity problems, with the likelihood that only 80 to 90% of infections are accurately diagnosed by this method. In addition, the procedure is relatively costly and has an approximately 48-h turnaround time.

Availability of a fairly rapid chemical-based assay or enzyme immunoassay (EIA) for detection of *Neisseria gonorrhoeae* would provide an alternative to culture and aid in efforts to control gonorrhea. Gonozyme (Abbott Laboratories) is an alternate method for the detection of gonorrhea and is commercially available. The system is based on solid-phase immunoassay for detection of gonococcal antigen in swabs collected from the urethra or cervix. Herein we would like to present an evaluation of this method for diagnosis of gonorrhea in patients attending two sexually transmitted disease clinics. The Gonozyme method was compared directly with culture.

MATERIALS AND METHODS

Patient population. This study was performed in two sexually transmitted disease clinics; one in Boston, Mass. (53 men and 155 women) and the other in Richmond, Calif. (64 men and 86 women). Urethral swabs or endocervical specimens were collected from patients attending these clinics and were transported to a separate laboratory for Gonozyme testing.

Specimen collection and cultural methods. Endocervical and urethral specimens were collected using cotton swabs. The swabs were inserted into the appropriate orifice, rotated, removed, and then streaked directly onto TM medium and placed into a specimen storage solution for the Gonozyme test. For some assays performed in Boston, these swabs were also streaked onto a chocolate agar (CA) plate before insertion into the Gonozyme transport medium. Presumptive identification of *N. gonorrhoeae* was based on compatible colonial morphology, oxidase reaction, and demonstration of gram-negative diplococci and was confirmed by appropriate sugar utilization patterns.

Gonozyme procedure. The principles of this test are as follows. (i) The clinical specimen is added to a specially treated bead which adsorbs gonococci and gonococcal antigens. (ii) After being washed to remove unbound material, the bead is exposed to rabbit antigonococcal antibody. (iii) Unbound rabbit antibody is removed by washing, and goat antibody to rabbit immunoglobulin G, conjugated with horseradish peroxidase, is added. (iv) After a final washing, a peroxidase substrate is added, and a visible color reaction takes place if the specimen is positive. The intensity of the color reaction can be quantitated with a spectrophotometer and will reflect the amount of peroxidase bound to the bead. The amount of peroxidase bound depends on the amount of bound rabbit antibody, which in turn is a reflection of the adherent gonococcal antigen.

The Gonozyme reagents and equipment were supplied by Abbott Laboratories. Test kits included negative and positive controls that were included in each run. The swabs arrived in the laboratory immersed in the storage reagent (0.2 ml). Specimen dilution buffer (1.0 ml) was added for 5 min before processing. The specimens were then vortexed for 10 to 15 s, and excess fluid was removed from the swabs by rotating them against the side of the tubes. The specimens (0.2 ml) were then added to wells in plastic plates. One positive and three negative controls were processed in parallel. A Gonozyme-treated bead was submerged in each well. Sealing the wells with an adhesive-backed card, a 15-min incubation in a 37°C water bath, aspiration of well contents, and four (15 ml) washes in water with Abbott Laboratories Pentawasher II followed this and each of the two subsequent fluid additions: (i) rabbit antibody to *N.*

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TABLE 1. Diagnosis of gonococcal infection of the male urethra by Gonozyme and culture

Gonozyme results	Culture results	
	Positive	Negative
Positive	70	0
Negative	5	42

gonorrhoeae (2), 0.20 ml per well; (ii) goat antibody to rabbit immunoglobulin G conjugated with horseradish peroxidase, 0.20 ml per well.

Finally, the beads were transferred to 5-ml reaction tubes. Freshly prepared enzyme substrate (orthophenyline diamine [2.50 mg/ml] in citrate-phosphate buffer containing 0.02% hydrogen peroxide [0.30 ml]) was added to each tube. The reaction proceeded for 10 min and was stopped by adding 1 ml of 1 N HCl.

Absorbance was read at 492 nm on a Quantum spectrophotometric analyzer. The machine was blanked using a substrate blank tube. Absorbance readings of >0.150 plus the mean absorbance of three negative controls were considered positive. All samples with absorbance readings of ≥0.150 and ≤0.300 were retested. Retest results were considered valid. Fisher's exact test was used for comparison of results.

RESULTS

Gonococcal urethritis in males. The results obtained for 64 men tested in Richmond and 53 tested in Boston were pooled because similar results were obtained. Data are presented in Table 1. The Gonozyme proved to be a relatively efficient method of detecting gonococcal antigen in this high-risk population. There was an overall culture-positive prevalence of 64.1% (75 of 117), and 70 of the 75 culture-positive men had positive Gonozyme tests, for a sensitivity of 93.3%. There were no false-positive reactions; thus, the specificity and predictive value of the positive Gonozyme reaction were 100%.

Cervical gonorrhea. There were differences in results obtained for women tested in Boston versus those tested in Richmond, and the separate results are presented in Table 2. The sensitivity of the Gonozyme test appeared to be markedly affected by multiple use of the same swab. When swabs were used for two bacterial cultures (TM and CA) before being placed in the Gonozyme transport system, the sensitivity fell from the 70% (17 of 24; Table 2, example A) observed when TM was the only medium to 38.5% (5 of 13; Table 2, example B; $P = 0.048$). Similarly, among 40 other women who had two samples tested (Table 2, example D), the sample that was inoculated directly into the Gonozyme transport medium was positive more often (64% [7 of 11]) among women who had positive TM cultures than a sample that was inoculated onto TM agar and CA before it was placed in Gonozyme transport medium (18% [2 of 11]; $P = 0.036$).

The overall prevalence of gonococcal infection was somewhat higher in the Richmond women (44.2% versus 31%), and the extra cultural step was excluded (Table 2, example C). The overall agreement between culture and Gonozyme assay was 93% (80 of 86), and the Gonozyme assay had a sensitivity of 86.8% as compared with culture (33 of 38). This sensitivity was higher than that of the same test in Boston (70%). False-positives occurred at a relatively low rate in both Boston and Richmond, with specificity being ca. 97 to 98% in both settings.

TABLE 2. Diagnosis of gonococcal infection of the cervix by Gonozyme and culture

Gonozyme results	Culture results	
	Positive	Negative
(A) Boston; one bacterial culture; $n = 68$ (patient → TM → tube)		
Positive	17	1
Negative	7	43
(B) Boston; two bacterial cultures; $n = 47$ (patient → TM → CA → tube)		
Positive	5	2
Negative	8	32
(C) Richmond; one bacterial culture; $n = 86$ (patient → TM → tube)		
Positive	33	1
Negative	5	47
(D1) Boston; two bacterial cultures; $n = 40$ (patient → TM → CA → tube)		
Positive	2	0
Negative	9	29
(D2) Boston; no bacterial cultures; $n = 40$ (patient → tube)		
Positive	7	0
Negative	4	29

DISCUSSION

In this high-risk population, it is clear that the Gonozyme test is technically capable of detecting gonococcal antigen in the great majority of patients currently infected with *N. gonorrhoeae*. It is obvious that the swabs used for the EIA should be placed directly into the Gonozyme transport system and not used for cultures. If cultural duplication is required, it should be with separate swabs, as the loss of material from the swab during the streaking procedure markedly affects the sensitivity of the Gonozyme assay.

Despite some reasonably good results obtained in the evaluation of this system, it is not clear what role it can play in the routine diagnosis of gonorrhea. For example, for symptomatic men the test appears to be essentially equivalent to a Gram stain, providing a sensitivity of ca. 93.3% and a specificity of 100%. However, the Gram stain is a relatively inexpensive test which provides a rapid result, and thus, it is unlikely to be replaced by an assay such as the Gonozyme for routine screening or first evaluation of symptomatic men with urethritis.

In women with cervical gonorrhea, with the best results obtained (Richmond series), the sensitivity of the test is ca. 87%, a rate which might not be acceptable in screening high-risk populations such as those seen in this sexually transmitted disease clinic. When gonococcal cultures are done on a routine basis, they are relatively inexpensive. A technician can read several hundred cultures a day, and EIA will be more expensive. In many hospital or private practice set-

TABLE 3. Results expected using Gonozyme and culture in screening women with a 1% prevalence of cervical gonorrhea

Gonozyme results	Culture results	
	Positive	Negative
Positive	87	205
Negative	13	9,695

TABLE 4. Routine use of Gonozyme in diagnosing gonorrhea

Gonozyme results	TM culture results	
	Positive	Negative
(A) Male urethra; n = 151		
Positive	43	1
Negative	2	105
(B) Cervix; n = 171		
Positive	20	13
Negative	3	135

tings, cultures are not done regularly enough to yield optimal results and, owing to increased administrative and other costs, are relatively expensive. Thus, one might suggest that the Gonozyme assay could play a role in screening for gonococcal antigen in cervical secretions in relatively low-risk populations. However, if the profile of sensitivity and specificity obtained in this study is projected into a relatively low-risk population, the projections do not suggest that it will be particularly useful. For example, in screening the high-risk women in both Boston and Richmond, the specificity of the test was ca. 97%. Projection of our "best case" analysis (Richmond data, with a sensitivity of 86.8% and a specificity of 97.9%) into a population with a 1% prevalence of gonorrhea yields the results presented in Table 3. Screening 10,000 women with a gonococcal infection rate of 1% would result in correct identification of 87 of 100 infected women, missing 13. Of greater concern is the fact that 205 false positives would result from this test; thus, the predictive value of a positive Gonozyme test would only be 87 of 292, or 29.8%. The fact that the assay must be performed on a specimen collected during a pelvic examination is also a mitigating factor, because one might as well do a culture. If the tests are used to screen women who would then have follow-up cultures, it is clear that many women with negative cultures would be brought back for repeat pelvic examinations, and there would be concomitant psychological trauma associated with the reason for the reexamination. The predictive value of the positive test is so low that it is clear that presumptive treatment could not be initiated purely on the basis of a positive Gonozyme test in a low-risk population. In addition, in the high-risk venereal disease clinic populations, the predictive value of a negative test for women was only about 90%; thus, there is no certainty that gonorrhea could be ruled out by a negative Gonozyme test.

There is an obvious caveat to this analysis. The gold standard which we must use is culture, which is recognized to be <100% sensitive; thus, there is a possibility that a Gonozyme false-positive could in fact be a false-negative result of culture. Perhaps further evaluation with repeat cultures and fluorescent antibody detection of gonococcal antigen in cervical secretions would allow greater confidence in the positive Gonozyme results. Currently, decisions must be made based on available data. We compared EIA with culture done on site by experienced staff. Perhaps the EIA may be more useful in transport of specimens when on-site cultures are not done.

After the experimental evaluation of the Gonozyme test was completed, the Contra Costa County Health Department performed an in-house evaluation in their laboratories. The results (Table 4) are consistent with those obtained in the initial evaluation. The EIA performed well in the case of males. There was 98% (148 of 151) agreement with TM; sensitivity of the test was 95.6% (43 of 45), and specificity was 99.1% (105 of 106). Performance was not as good when cervical specimens were tested. Agreement with TM was 90.6% (155 of 171); the sensitivity was 87% (20 of 23), and the specificity 91.2% (135 of 148). False-negative rates of 13.0% (3 of 23) and false-positive rates of 8.8% (13 of 148) were considered too high for routine use of EIA in place of culture.

Culture-positive, EIA-negative specimens had a range of two colonies on the plate to 4+ growth. Thus, the number of CFU in the specimen does not appear to be the sole determinant of EIA positivity.

The published experience with this test is not extensive, but some similar results have been obtained elsewhere. For example, Aardoom et al. also found the Gonozyme assay to be a particularly useful test for men with urethritis, but they obtained less satisfactory results for women (1). Danielsson et al. had somewhat less success in testing urethral specimens; with both men and women they found a sensitivity of 97.8% (3). Although the numbers were small, their results were slightly better for women than for men.

In summary, the Gonozyme test appears to be capable of detecting gonococcal antigen in genital tract discharges of men and women. Its sensitivity, specificity, and predictive value are similar to those for the Gram stain in the case of symptomatic men with a high prevalence of gonococcal urethritis, and the test appears inadequate to replace culture for women. It is clear that some improvement in this test will be required before it can be recommended for routine screening.

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