A Solid-Phase Enzyme Immunoassay in Detection of Cervical Gonorrhea in a Low-Prevalence Population

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An evaluation of a solid-phase enzyme immunoassay (Gonozyme) for detection of gonococcal antigen in cervical swab specimens was undertaken in 504 asymptomatic women undergoing routine gynecologic examination. The immunoassay was positive in all seven women with culture-proven gonorrhea. Negative immunoassay results were obtained in 482 of the 497 women with negative cultures (97.0 percent specificity). The enzyme immunoassay's performance equals or exceeds that of other rapid alternative methods of diagnosing cervical gonorrhea such as Gram stain or limulus lysate assay. Its usefulness, however, is limited by less than 100 percent specificity, particularly in low-prevalence populations. More studies are needed to ascertain the performance of both this immunoassay and modified Thayer-Martin culture techniques in diagnosing cervical gonorrhea in low-prevalence populations.

Gonorrhea is a major health problem in the United States. Although there were 990,864 cases reported in 1981, the total number was probably twice that because of underreporting. In the period between 1960 and 1980, reported cases of gonorrhea climbed from 258,933 to 1,004,029.[1]

Family physicians are integrally involved in the detection and treatment of gonorrhea. Private physician offices are the source of 20 percent of the female cultures for gonorrhea performed by government agencies.[1] The number performed privately is not known.

In the 1960s, Thayer and Martin[2,3] developed and modified a selective medium that made culture of Neisseria gonorrhoeae possible. Since that time, the modified Thayer-Martin culture medium has been the only satisfactory method of detecting gonorrhea in asymptomatic women. However, because the culture requires a 24- to 48-hour incubation period, more rapid, alternative methods have been sought.

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In 1982 Abbott Laboratories introduced a Food and Drug Administration-approved solid-phase immunoassay with the trade name Gonozyme for the detection of N gonorrhoeae in urogenital swab specimens. The specimen is collected in a manner similar to that for a Thayer-Martin culture, and the results can be obtained within two hours. Because the immunoassay is currently being used in community laboratories, it is appropriate that family physicians become acquainted with it. The immunoassay was tested in a low-prevalence population.

Methods

The Edward W. Sparrow Hospital Family Practice Residency consists of 25 residents and six faculty members. It serves a private patient population of 8,363 patients in 3,442 families. In addition, it has contracted with the Ingham County Health Department Family Planning-Prenatal Clinics to provide services to an additional 3,383 patients.

Between February 4 and March 15, 1983, all female patients of these populations being tested for gonorrhea by Thayer-Martin cultures of cervical specimens were also tested for gonorrhea using the enzyme immunoassay. The women included in this study were asymptomatic, and the procedures were undertaken as part of prenatal, family planning, or other routine gynecologic examination. The immunoassay test was performed for comparison purposes only, and no therapeutic decisions were made based on the test results.

Speculum visualization of the cervix was performed by the patient's physician. A Dacron swab was inserted into the cervical os, rotated gently, and smeared onto a glass slide for endocervical Papanicolaou smear. The same swab was reinserted into the os, left in place for 5 seconds, and streaked directly onto a modified Thayer-Martin medium in a large Z pattern. The same swab was reinserted into the os a third time, rolled gently, and placed into a transport tube containing storage reagent.

The plates were placed in a candle jar at 37° C and delivered within 8 hours to the State of Michigan Public Health Laboratories, or to the E.W. Sparrow Hospital Clinical Laboratories, where they underwent processing. The transport tubes were stored in a refrigerator at 2° to 8° C until transported to the E.W. Sparrow Hospital Clinical Laboratories where they underwent processing within five days.

After incubation at 37° C in five percent carbon dioxide atmosphere for 24 to 28 hours, the plates were examined for N gonorrhoeae based on the presence of characteristic colonial morphology, positive oxidase activity, and Gram-negative diplococci. The presumptively identified colonies were confirmed by the Phadebact Gonococcus Test, a coagglutination procedure.

The procedure used in this assay was that developed by Abbott Laboratories and marketed as the Gonozyme Diagnostic Kit. In the test, the swab specimen is incubated with a specially treated bead that adsorbs gonococcal antigen. After washing, the bead is exposed to rabbit anti-gonococcal antibody. After another washing to remove unbound rabbit antibody, the bead is incubated with a goat antibody to rabbit immunoglobin G. The goat antibody has been conjugated with horseradish peroxidase. After a final washing, the bead is incubated with o-phenylenediamine for 10 minutes. The peroxidase causes a yellow-orange color to develop in proportion to the quantity of N gonorrhea antigen adsorbed to the bead.

The absorbance of negative controls (supplied by the manufacturer) and specimens is measured by spectrophotometer at 492 nM. The cutoff value is the mean of the negative controls plus the factor 0.190. Specimens giving absorbance values equal to or greater than the cutoff value are considered positive for N gonorrhoeae.

Results

The results are shown in Table 1. There were seven patients with positive endocervical cultures. All seven cultures also were positive by immunoassay. Four hundred ninety-seven patients had negative cultures. Of these cultures, 482 were

Table 1. Comparison of Results Obtained With Thayer-Martin Culture and Gonozyme

	Culture positive	Culture negative
Gonozyme positive	7 true positive	15 false positive
Gonozyme negative	0 false negative	482 true negative

Incidence 7/504 = 1.40%

Sensitivity TP/(TP+FN) = 7/(7+0) = 100%

Specificity TN/(TN+FP) = 482/(482+15) = 97.0%

Positive predictive value TP/(TP+FP) = 7/(7+15) = 31.9%

negative and 15 were positive by immunoassay.

Using the Thayer-Martin culture as the standard, a sensitivity of 100 percent was obtained by immunoassay. The small number of positive cultures does not allow, however, definite conclusions about the test's true sensitivity. Again, using the Thayer-Martin culture as the standard, the specificity of the immunoassay was 97.0 percent (P = .001). The predictive value of a negative immunoassay result in the study population was 100 percent. The predictive value of a positive immunoassay result in this population was 31.9 percent.

The distribution of sample spectrophotometric absorbance about the cutoff value is shown in Figure 1. It is interesting to observe that had the factor added to the negative control mean been 0.230 rather than 0.190, the immunoassay would have performed identically with the Thayer-Martin cultures. However, for this experiment, the manufacturer's instructions were strictly observed.

Comment

The modified Thayer-Martin culture is the recommended technique for diagnosis of gonorrhea in women.[4] Using the Thayer-Martin culture as the standard, alternative methods have been investi-

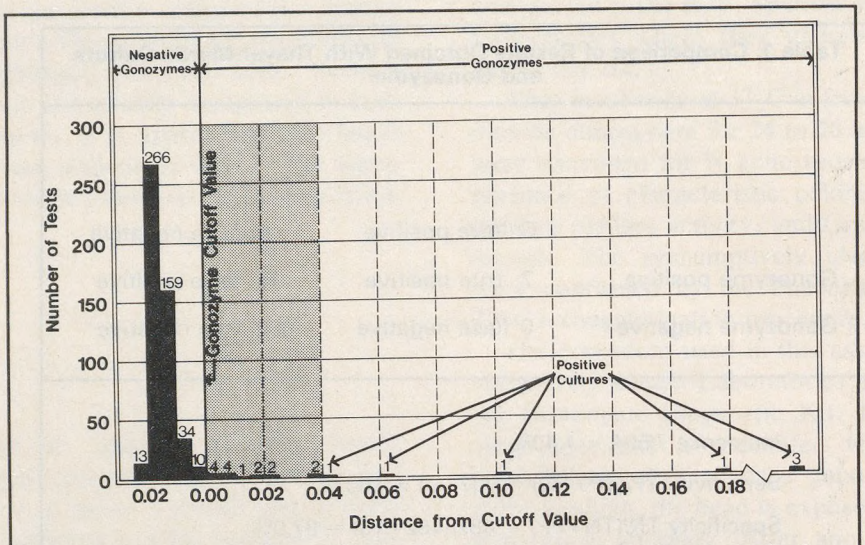

Figure 1. Distribution of spectrophotometric absorbance of the Gonozyme test

gated (Table 2).[5-12]

The literature contains four evaluations of the Gonozyme immunoassay.[9-12] All four have been performed in high-prevalence populations (usually sexually transmitted disease clinics). It should be pointed out that the spectrophotometric cutoff value in these studies was different than that used in this study.

The efficacy of the Thayer-Martin culture is not perfect. As the technique involves actually culturing the organism, with appropriate confirmation tests, its specificity approaches 100 percent. However, studies in high-prevalence populations have shown the sensitivity of a single endocervical culture to be 80 percent to 90 percent.[13,14] There are no studies assessing the culture's sensitivity in low-prevalence populations.

One problem with evaluating the Thayer-Martin culture is the lack of a good method with which to compare it. All of the techniques available are dependent upon the number of organisms present in the inoculum. Therefore, any investigation (including serial cultures) that compares two methods will be complicated by this lack of independence.

It should be emphasized that the 97.0 percent specificity obtained for the immunoassay assumed a 100-percent sensitivity for Thayer-Martin. As the culture's sensitivity is not 100 percent, a pro-

Table 2. Comparison of Methods for Diagnosing Gonococcal Cervicitis

Study	Technique	n	Prevalence (%)	Sensitivity (%)	Specificity (%)	Comment
Lossick	Gram stain	8,537	19.6	70.4	97.0	Compared Gram stain with culture
Spagna	Limulus lysate	40	45.0	94.4	100.0	Compared 1:800 dilution of cervical secretions with culture
Young	Limulus lysate	66	36.4	62.5	90.5	Compared 1:100 dilution of cervical secretions with culture
Hainer	Limulus lysate	48	8.3	100.0	56.8	Compared 1:2000 dilution of cervical secretions with culture
Aardoom	Enzyme immunoassay	54	27.8	86.7	89.7	Used a cutoff value of 0.15
Danielsson	Enzyme immunoassay	150	7.3	90.9	100.0	Cutoff value not stated
Burns	Enzyme immunoassay	368	25.0	88.5	94.3	Used a cutoff value of 0.15
Schachter	Enzyme immunoassay	171	13.5	87.0	91.2	Used a cutoff value of 0.15
Present study	Enzyme immunoassay	504	1.4	100.0	97.0	Used a cutoff value of 0.19

portion of the cases treated as immunoassay false-positives may in fact be true-positives. An 80- to 90-percent sensitivity for Thayer-Martin makes it unlikely that the immunoassay's specificity is biased to a great extent; however, this matter deserves further investigation.

Another issue that deserves mention is the situation in which asymptomatic women should be tested for gonorrhea. None of the current screening guidelines recommend inclusion of testing asymptomatic women as part of the routine examination[15-18]; there is evidence, however, that such testing is performed.[19] Yorke and colleagues[20] have estimated that the incidence of gonorrhea is 20 percent lower because of a national screening effort, although this figure has been questioned more recently.[21] There is little doubt that the disease has enormous economic consequences.[22]

As the role of screening is not clear at this time, the family physician must consider thoughtfully what he or she seeks to obtain in a test for gonorrhea, and must weigh carefully the advantages and disadvantages, the costs and benefits of the diagnostic method.

The Gonozyme immunoassay is more sensitive and more specific than either Gram stain or limulus lysate assay in diagnosing cervical gonorrhea. However, when compared with Thayer-Martin culture, the 97.0 specificity of the immunoassay still proves to be a serious drawback, especially in a low-prevalence population.[23] Further investigation of its capabilities and of the impact of detecting gonorrhea in asymptomatic women is necessary. At present, the immunoassay may find its greatest value in the venereal disease clinic or emergency department.

Acknowledgments

The authors wish to acknowledge the assistance of the State of Michigan and Ingham County Departments of Public Health and Abbott Laboratories.

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