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Validity of an Enzyme Immunoassay for Detection of *Neisseria gonorrhoeae* Antigens

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An enzyme immunoassay (EIA; Gonozyme, Abbott Laboratories) for the antigenic detection of *Neisseria gonorrhoeae* in endocervical or urethral specimens was evaluated. EIA results were compared with results of conventional culture tests for *N. gonorrhoeae*. Specimens from 208 males (113 culture positive) and 252 females (72 culture positive) were tested. The sensitivity and specificity of EIA for specimens from males were 97.3 and 95.8%, respectively. The sensitivity and specificity of EIA for specimens from females were 79.2 and 87.2%, respectively.

Gonorrhea ranks first among reported communicable diseases in the United States. More than 1,000,000 cases were reported in 1979, and it is estimated that an additional 600,000 to 1,000,000 cases were not reported in that year (3). Local records indicate that 4,024 cases of gonorrhea were reported in Erie County, N.Y., in 1982. As calculated from the 1980 census figure of 1,015,472 Erie County residents, the incidence of gonorrhea in Erie County was 396 per 100,000. The number of reported cases in Erie County has remained essentially constant since 1979.

Efforts to control the spread of gonorrhea include bacteriological screening of asymptomatic females and attempts to identify and treat sexual contacts of individuals with gonorrhea. Unfortunately, these efforts are hampered by the limitations of current laboratory methods for confirming the diagnosis of gonorrhea. Isolation of *Neisseria* spp. from clinical specimens and subsequent identification of the species of these isolates by biochemical and growth characteristics requires at least 2 days. This can be reduced to 24 h if coagglutination or fluorescent-antibody tests are used for the definitive identification of *Neisseria gonorrhoeae* (8, 14). Regardless of the method used to identify *Neisseria* isolates by species, the sensitivity of a single endocervical culture for diagnosing gonorrhea in females appears to be less than 90% (2, 13, 15). Clearly, a rapid, sensitive, and specific technique for detecting *N. gonorrhoeae* in clinical specimens would greatly assist public health efforts to control the spread of gonorrhea.

An enzyme immunoassay (EIA; Gonozyme, Abbott Laboratories, North Chicago, Ill.) for detecting antigen(s) of *N. gonorrhoeae* in urethral (males) or endocervical (females) swab specimens was recently introduced. The product is designed to evaluate clinical specimens for the presence of *N. gonorrhoeae* within 2 h. Our experience with this EIA is presented in this report.

MATERIALS AND METHODS

Specimen collection. The patient population consisted of individuals who came from treatment to either the Erie County Department of Health, Sexually Transmitted Disease Clinic, or the medical emergency room at the Erie County Medical Center. Specimens were collected from asymptomatic and symptomatic females (252) and from symptomatic males (208), but not from asymptomatic males. Specimens from asymptomatic females were collected exclusively at the Sexually Transmitted Disease Clinic. Urethral (male) or endocervical (females) specimens were taken in duplicate from each patient on two separate swabs. The first swab was used for Gram stain, culture, or both; the second swab was used for the EIA.

Bacteriological culture. Swab specimens were promptly streaked on Martin Lewis or modified Thayer-Martin agar plates at the site of specimen collection, and the plates were transported to the laboratory. The plates were incubated at 36°C in an atmosphere of 95% air and 5% CO_2. Cultures were examined daily for 4 days. Suspect colonies were Gram stained and tested for the presence of cytochrome oxidase. Gram-negative diplococci that produced cytochrome oxidase were confirmed as *N. gonorrhoeae* by their capability to dissimilate dextrose, their failure to dissimilate maltose and sucrose, and their lack of growth at room temperature on chocolate agar (12, 18).

EIA specimen processing and assay procedure. Immediately after swab specimens for the EIA were secured, they were placed into 100 μl of a specimen storage reagent (Abbott Laboratories) and refrigerated (2 to 8°C) for up to 5 days before testing. Before being tested, swabs were removed from specimen storage reagent and immersed in 1.0 ml of specimen dilution buffer (Abbott Laboratories) for 2 min, mixed vigorously for 20 s, and then squeezed to remove excess fluid. The EIA was performed as directed by the manufacturer. Briefly, 0.2 ml of the controls or of specimens from patients was incubated with treated beads (capable of binding antigens of *N. gonorrhoeae*) in a 37°C water bath for 15 min. Unbound material was aspirated, and the beads were washed with distilled water in the Abbott Pentawash System. Anti-*N. gonorrhoeae* rabbit serum (0.2 ml) was added to each bead. The beads were incubated at 37°C for 15 min and were washed by aspiration. Anti-rabbit immunoglobulin goat serum coupled with horseradish peroxidase (0.2 ml) was added to each bead and incubated at 37°C for 15 min. Then unbound material was aspirated, and the beads were washed. Each bead was treated with 0.3 ml of freshly prepared *o*-phenylenediamene reagent and incubated for 10 min at room temperature, and 1 ml of 1.0 M HCl was added to stop the reaction. Absorbance at 492 nm was determined with a Quantum (Abbott Laboratories) photometric analyzer.

Interpretation of results. Three negative and one positive control specimens were run with each assay. Abbott Labora-

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tories has established the cutoff for positivity (COP) as the mean absorbance of the three negative controls plus 0.190. Specimens with absorbance values above the COP are interpreted as positive by the EIA, and specimens with absorbance values below the COP are considered negative. EIA results for specimens with absorbance values within ±13% of the COP were considered inconclusive until they were retested. The EIA was evaluated as a diagnostic test for gonorrhea by comparing EIA results with results of bacteriological cultures. EIA results were categorized as true-positive (TP) (culture and EIA positive), true-negative (TN) (culture and EIA negative), false-positive (FP) (culture negative, EIA positive), and false-negative (FN) (culture positive, EIA negative). Sensitivity, specificity, and positive and negative predictive values were calculated as follows: sensitivity = [TP/(TP + FN)] × 100; specificity = [TN/(FP + TN)] × 100; positive predictive value = [TP/(TP + FP)] × 100; negative predictive value = [TN/(FN + TN)] × 100.

The reproducibility of the EIA was evaluated by testing specimens from the first 188 patients in duplicate and calculating the standard deviation for a single EIA determination (6) by the equation $s = \sqrt{\Sigma (d^2)/2N}$, where s is the standard deviation of a single EIA determination, d is the difference between the absorbances of paired specimens, and N is the number of paired specimens.

RESULTS

The results of the EIA compared with conventional culture results for 208 male specimens are presented in Table 1. The sensitivity, specificity, and positive and negative predictive values all exceeded 95%.

EIA results for 252 specimens from females are compared with conventional culture results in Table 2. Although specificity was 87.2%, the sensitivity was only 79.2%.

EIA absorbances for all specimens were evaluated for their distribution around the COP (Table 3) because it appeared that the absorbances of FP endocervical specimens were considerably lower than those of TP endocervical specimens. Of the 208 male specimens, 5 (2.4%) had absorbances within ±25% of the COP; EIA and culture results were concordant for 3 (3 TP) and discordant for 2 (2 FN) of these 5 specimens. In all, 2 of 3 FN (66.7%), 0 of 4 FP, 3 of 110 TP (2.7%), and 0 of 91 TN male specimens were within 25% of the COP.

Endocervical specimens with discordant EIA and culture results frequently (12 of 38) had absorbances within ±25% of the COP. A much lower proportion (12 of 214) of endocervical specimens with concordant EIA and culture results had absorbances in this range. Of the 252 female specimens, 24 (9.5%) had absorbances within ±25% of the positive cutoff; EIA and culture results were discordant in 12 (8 FP, 4 FN) and concordant in 12 (2 TP, 10 TN) of these 24 specimens. In all, 8 of 23 FP (34.8%) and 4 of 15 FN (26.7%) specimens had absorbances within ±25% of the COP, whereas only 2 of 57 TP (3.5%) and 10 of 157 TN (6.4%) specimens had absorbances in this range.

TABLE 1. Validity of EIA (Gonozyme) compared with conventional culture for 208 male specimens

EIA results[a]	Culture results	
	Positive	Negative
Positive	110 (TP)	4 (FP)
Negative	3 (FN)	91 (TN)

[a] Sensitivity, 97.3%; specificity, 95.8%; positive predictive value, 96.5%; negative predictive value, 96.8%.

TABLE 2. Validity of EIA (Gonozyme) compared with conventional culture for 252 female specimens

EIA results[a]	Culture results	
	Positive	Negative
Positive	57 (TP)	23 (FP)
Negative	15 (FN)	157 (TN)

[a] Sensitivity, 79.2%; specificity, 87.2%; positive predictive value, 71.3%; negative predictive value, 91.3%.

The reproducibility in the EIA procedure was determined by testing specimens from the first 188 patients in duplicate and calculating the standard deviation for a single EIA determination (6). For analysis, specimens were divided into two groups, those with mean absorbances of ≤0.50 (153 paired specimens) and those with absorbances between 0.51 and 1.999 (35 paired specimens). The standard deviations for a single EIA determination were 0.013 and 0.069 for the ≤0.5 and 0.51 to 1.999 groups, respectively.

DISCUSSION

The sensitivity (97.3%) and specificity (95.8%) of the EIA for male specimens would suggest this method as a suitable alternative to culture for establishing definitive evidence of gonorrhea. In two of the four males with FP EIAs, intracellular and extracellular gram-negative diplococci were seen in urethral exudates. Thus, the adjusted number of FP EIAs would be two since two of the four specimens fitting our definition of FP most likely represented problems associated with recovery of gonococci in culture rather than errors of the EIA. If these two specimens are considered TP rather than FP, the sensitivity and specificity of the EIA become 97.4 and 97.8%, respectively.

The sensitivity and specificity of the EIA for specimens from females were 79.2 and 87.2%, respectively. Chart review of 18 of the 23 females (charts for 5 females were unavailable) whose specimens yielded FP EIA results revealed that at the time of specimen collection, 3 patients were taking antibiotics known to inhibit growth of *N. gonorrhoeae* and 2 other patients were being evaluated for efficacy of treatment for a recent *N. gonorrhoeae* infection. Presumably, tests of cure patients could harbor breakdown

TABLE 3. Evaluation of EIA absorbance for proximity to COP

Population[a]	Total	No. within ±25% of COP[b]
Males		
FP	4	0
FN	3	2
TP	110	3
TN	91	0
Females		
FP	23	8
FN	15	4
TP	57	2
TN	157	10

[a] FP, TP, and TN indicate interpretation of the EIA based upon comparison with conventional culture for *N. gonorrhoeae*.

[b] The COP was determined by adding 0.190 to the mean absorbance of three negative control specimens.

products of *N. gonorrhoeae* which are recognized immunologically by the EIA but are not viable. Similarly, it is questionable whether *N. gonorrhoeae* could be recovered from specimens of patients receiving antibiotics. If these 5 patients are eliminated from the study, the sensitivity of the EIA would be the same (79.2%), and the specificity would become 89.7%. At this validity level it is doubtful that laboratories would adopt this diagnostic modality for establishing definitive evidence of gonorrhea in females.

To our knowledge, there have been two previously published clinical evaluations of the Gonozyme EIA (1, 5). Danielsson et al. (5) reported sensitivity and specificity of 90.9 and 100%, respectively, for females and 83.3 and 94.3%, respectively, for males. Our experience with the EIA differed from that of Danielsson and co-workers. A possible reason for this difference is that their study population included only 11 females and 12 males with positive *N. gonorrhoeae* cultures.

For 52 men with symptoms of urethritis, Aardoom et al. (1) reported a sensitivity and specificity of 100% with the EIA procedure. For specimens from males, the sensitivity and specificity that we attained with the EIA were similar to those reported by Aardoom et al. In the same investigation, Aardoom et al. reported 86.7% sensitivity and 89.7% specificity for 54 female contacts of individuals suspected of having gonorrhea and 91.7% sensitivity and 96.7% specificity for 102 female prostitutes who regularly attended a clinic for sexually transmitted diseases. The specificity of EIA which we obtained with female specimens was similar to that obtained with the gonorrhea contact group of Aardoom et al. but was lower than that seen in the prostitutes. The sensitivity of EIA that we obtained with female specimens was lower than that obtained in either of the groups of Aardoom et al. for reasons that are not entirely clear.

The low sensitivity (79.2%) of the EIA for endocervical specimens may be due to a subpopulation of females with relatively few recoverable gonococci (9). Clearly, the number of gonococci required for detection by culture (theoretically 1 viable organism) is less than the number required for detection by the EIA. Thus, specimens from individuals with sufficient numbers of gonococci for positive culture but insufficient for a positive EIA would yield FN EIA results. The method for specimen collection may have contributed to the high number of FN EIAs since two swabs, the first for culture and the second for the EIA, were taken from each patient. It is probable that the second swab would contain fewer gonococci than the first and would therefore be less likely to give a positive EIA result.

The high sensitivity of EIA for urethral specimens from symptomatic males suggests that the subpopulation of males with numbers of recoverable gonococci intermediate between that required for positive culture and that required for positive EIA either is very small or was excluded from the study because only symptomatic males were tested.

Another possible explanation for the substantial difference in the sensitivity of the EIA for specimens from males and females is the competition by antigen(s) (microorganisms other than *N. gonorrhoeae*) in the female genitourinary tract. According to EIA methodology, a suspension prepared from the specimen of the patient is incubated with a treated bead; gonococcal antigens, if present in the suspension, adsorb onto the bead. The bead, with adsorbed materials, is subsequently incubated with anti-*N. gonorrhoeae* rabbit serum. If nongonococcal antigens adsorb onto the bead, they may compete with gonococci for binding sites on the treated bead, thus inhibiting adsorption of gonococcal antigens. If the anti-*N. gonorrhoeae* rabbit serum does not recognize these adsorbed nongonococcal antigens, the resultant EIA will be negative, despite the presence of gonococcal antigens in the original suspension.

FP EIA results may be due either to the inability of organisms present in the specimen to grow on culture or to immunological recognition of antigen(s) from other organisms that cross-react with antigen(s) of *N. gonorrhoeae*. *N. gonorrhoeae* is a fastidious organism, and viability may be lost because of improper specimen handling (16, 17). Similarly, vancomycin, which is present in Martin-Lewis and Thayer-Martin agars, inhibits growth of vancomycin-sensitive strains of *N. gonorrhoeae* (11). The high positive predictive value of the EIA for males (96.5%) in contrast to that for females (71.3%), provides circumstantial evidence that improper specimen handling did not contribute significantly to FP EIA results. If improper specimen handling was a significant cause of FP EIA, males and females might be expected to have a similar proportion of FP EIA results since specimens from males and females were handled similarly. The role of growth inhibition by vancomycin in the culture media is more difficult to ascertain. If the incidence of these susceptible strains is the same in infections of males and females, then we could conclude that susceptible strains are rare because very few FP results were obtained with specimens from males. This reasoning is impaired by the choice of both symptomatic and asymptomatic females, compared with symptomatic males only, for study since it is well documented that asymptomatic infections in males are frequently due to strains that require arginine, hypoxanthine, and uracil (AHU strains) and that these AHU strains are highly susceptible to vancomycin (4, 7, 10, 11). Therefore, the role of inhibition of *N. gonorrhoeae* by vancomycin as a cause of FP EIA results is difficult to determine.

Lack of immunological specificity is the most reasonable explanation for the low positive predictive value of the EIA for specimens from females. Two observations support this view. First, the positive predictive value of the EIA was substantially higher for males (96.5%) than for females (71.3%); this suggests that certain antigenic determinants recognized by the EIA are present in specimens from females only. Surely the quantity and heterogeneity of the female genitourinary microbiota provides a far greater challenge to the specificity of immunological reagents than the comparatively sparse microbiota of the male urethra. Second, the photometric absorbance of a large proportion of FP female specimens was substantially lower than the absorbance of TP specimens. This suggests that the anti-*N. gonorrhoeae* rabbit serum binds weakly to nongonococcal antigens or to gonococcal antigens that cross-react with antigens of other microorganisms. The sensitivity and specificity of the assay for females might therefore be improved by carefully evaluating the antisera to determine and identify these antigens.

If the sensitivity and specificity of EIA for detection of *N. gonorrhoeae* in females can be improved, this assay could have a major impact on public health efforts to control the spread of gonorrhea. At present, however, one potential deterrent to using the EIA to identify *N. gonorrhoeae* is that organisms would not be available for assessing β-lactamase production.

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