Evaluation of Gonozyme, an Enzyme Immunoassay for the Rapid Diagnosis of Gonorrhea

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A new indirect enzyme immunoassay (EIA), Gonozyme (Abbott Laboratories), was assessed for rapid detection of gonococcal antigens. A correlation of optic density (OD) readings by EIA with colony counts of serial dilutions of Neisseria gonorrhoeae ATCC 19424 disclosed that EIA detected 10^3 CFU/ml at OD readings of 0.1 to 0.3, that EIA consistently detected ≥10^4 CFU/ml at OD readings of 0.6 to 1.3, and that concentrations of ≥10^5 CFU/ml were associated with OD readings of >2.0. The clinical usefulness of Gonozyme was evaluated by comparing results of EIA with those of Gram stain (GS) and culture for N. gonorrhoeae from urethral and endocervical swabs obtained prospectively in 886 randomly selected patients attending a clinic for sexually transmitted diseases. The patients evaluated included 83 female contacts of men with gonorrhea and 56 patients seen at the clinic for test of cure. In tests with 295 males, the sensitivities of GS and EIA were 91.3 and 97.1%, respectively, and both tests had specificities of >96%. In tests with 591 females, the sensitivities of GS and EIA were 51.4 and 96.4%, respectively (P < 0.0001, Z proportionality test), and the specificities were 98.7 and 86.5%, respectively (P < 0.0001). In tests with 61 females and 3 males, EIA was positive, whereas GS and cultures were negative for N. gonorrhoeae. Gonozyme is a highly sensitive method for rapid detection of gonococcal antigens. EIA is comparable to GS for males and more sensitive though less specific than GS for females. Possible reasons for the lower specificity of EIA for females are discussed. Due to its high negative predictive value for female contacts, EIA offers an alternative to epidemiological treatment of contacts before culture results.

Gonorrhea is currently prevalent in the United States in epidemic proportions, with nearly 1,000,000 cases reported annually (8). Prompt diagnosis of gonorrhea at the time the patient visits the clinic for sexually transmitted diseases (STD) is important to ensure appropriate specific treatment and to reduce the occurrence of complications such as pelvic inflammatory disease. In a recent study conducted at an STD clinic, 7.3% of women with gonococcal cervicitis who were not treated at the time of the initial visit developed pelvic inflammatory disease before the second visit (17). Although the culture results in that study were provided 48 h after the initial clinic visit, the mean interval between the first examination and follow-up treatment was 7 days. Diagnosis of gonococcal urethritis and cervicitis by Gram stain of the exudate is used most frequently and is often the only rapid diagnostic method used; unfortunately, the sensitivity of Gram stain for female patients is only about 50% (3, 12, 29).

Gonozyme (Abbott Laboratories, North Chicago, Ill.) is a new indirect enzyme immunoassay (EIA) which has been developed for rapid diagnosis of gonorrhea. In this EIA system, the non-antibody-modified surface of a bead binds gonococcal antigens present in the solution which is being tested. Gonococci present in the solution are rendered nonviable by the specimen preservative and dilution buffer included in the kit. The gonococcal antigens, in turn, are bound to rabbit antibody, and the latter is bound to enzymegoat antibody conjugate, which produces a color change read by a spectrophotometer.

We evaluated the minimum in vitro concentration of Neisseria gonorrhoeae which could be detected by Gonozyme by correlating serial dilutions of pure cultures of N. gonorrhoeae with the Gonozyme optical density (OD) reading of the serial dilutions. We determined the specificity of Gonozyme by testing it with pure cultures of various aerobic and anaerobic bacteria which can be found in the genital tract (21). Finally, we prospectively evaluated the clinical usefulness of Gonozyme for rapid detection of N. gonorrhoeae in swabs from male urethral and female endocervical secretions and compared the results of EIA with those of Gram stain and culture.

MATERIALS AND METHODS

In vitro evaluation. For the determination of the minimum concentration of N. gonorrhoeae detected by Gonozyme, we compared the results of plate counts of serial dilutions of N. gonorrhoeae ATCC 19424 with the corresponding dilutions of the organism in Gonozyme specimen dilution buffer (Abbott Laboratories). Because of the fastidious nature of N. gonorrhoeae, we used a modification of the gonococcal base broth described by Shockley et al. (25), containing Proteose peptone no. 3, 15 g; K_2HPO_4, 4 g; KH_2PO_4, 1 g; NaCl, 5 g; and soluble starch (Mallinckrodt, Paris, Ky.), 1 g; in 1,000 ml of distilled water. To 100 ml of this sterilized medium, 1 ml of Supplement B (Difco Laboratories, Detroit, Mich.) and 1 ml of a sterile 20% glucose solution were added. The confluent growth of an 18-h agar plate of N. gonorrhoeae was swabbed and suspended in 5 ml of gonococcal base broth by vigorous twirling and vortexing. Portions (0.5 ml) of this suspension were then used to inoculate each flask. We grew the cultures on a shaker to reduce clumping of cells in the broth.

Preliminary tests were done to establish the log phase of growth of N. gonorrhoeae in gonococcal base broth. Samples of the cultures grown in the above medium at 37°C on a rotary shaker were removed at 2-h intervals, and we found
that *N. gonorrhoeae* was in the log phase of growth between 2 and 6 h at cell concentrations of $10^6$ to $10^8$ CFU/ml. Consequently, we removed 1-ml samples of 5-h cultures of the organism for serial dilutions. Colony counts were prepared on chocolate agar plates which were incubated in a candle jar at 37°C for 24 to 48 h. The corresponding dilutions in Gonozyme specimen dilution buffer were processed by Gonozyme. Three separate determinations of the correlation between CFU of *N. gonorrhoeae* per milliliter and OD readings of Gonozyme were processed in duplicate.

The specificity of Gonozyme was evaluated by testing overnight cultures in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) of the following organisms: *Neisseria meningitidis* ATCC 13090, *Neisseria sicca* ATCC 9913, *Neisseria lactamica* ATCC 23970, *Neisseria mucosa* ATCC 19696, *Neisseria flavescens* ATCC 13120, *Neisseria subflava* ATCC 14799, *Gemella hemolysans* ATCC 10379, *Branhamella catarrhalis*, 16 clinical strains of *Staphylococcus aureus*, and 15 species of *Enterobacteriaceae* and *Pseudomonas*. The inoculum concentrations of these aerobes ($10^6$ to $10^8$ CFU/ml) were established by the plate count method. These suspensions were tested by Gonozyme as outlined for *N. gonorrhoeae*. We also tested the following anaerobic bacteria: *Bacteroides fragilis* ATCC 23745, six clinical isolates of *B. fragilis* which were not subspeciated, *Bacteroides melaninogenicus* ATCC 15033 and 15930, *Bacteroides bivius*, *Bacteroides distasonis*, *Bacteroides vulgatus* ATCC 8482, *Bacteroides thetaotaomicron* ATCC 29742, *Bacteroides ovatus*, *Fusobacterium necrophorum* ATCC 25286, *Fusobacterium lentum* ATCC 25559, *Bifidobacterium breve* ATCC 15700, *Veillonella parvula* ATCC 10790, *Pepstrostreptococcus anaerobius* ATCC 27337, *Pepstrostreptococcus micros* ATCC 33270, and *Peptococcus prevotii* ATCC 9321. These anaerobes were suspended in Gonozyme specimen dilution buffer to a concentration of $3 \times 10^8$ (no. 1 McFarland standard). Tenfold dilutions of the latter solution were processed by Gonozyme.

**Patients.** Urethral and endocervical swabs were randomly obtained from December 1982 through April 1983 from unselected patients attending the Municipal Social Hygiene Clinic, Chicago, Ill., the largest clinic for treatment of STD in the city. In the majority of men, urethritis with or without urethral discharge was the reason for the clinic visit. The women included patients with symptoms of urethritis or cervicitis and asymptomatic females who presented themselves for STD examination for various reasons. In our study, patients were grouped into three mutually exclusive categories: patients presenting for an STD examination regardless of symptoms, patients evaluated at the clinic for test of cure, and contacts. Patients evaluated for test of cure were those who had been treated within the previous 2 weeks for gonorrhea, based on a positive culture. Contacts were defined as individuals who were named as having had a sexual contact with a person who had been previously diagnosed at the STD clinic as having gonorrhea.

**Specimen processing.** Swabs were obtained from the urethral secretions of males and from the cervical exudate (by speculum examination) of females. Two swabs in sequence were obtained from each patient. The first swab from both males and females was a standard cotton-tipped swab which was used to prepare a smear of the exudate. The second swab was provided by Abbott Laboratories (STD-PEN for males and STD-EZE for females). This swab was first streaked at the clinic on modified Thayer-Martin agar (Scott Laboratories, Fiskeville, R.I.), and then placed into a vial containing 2 drops of Gonozyme storage reagent (Abbott Laboratories). The modified Thayer-Martin plates were immediately put into a candle jar and transported to our research laboratory within 3 h for incubation at 37°C for up to 48 h. Identification of *N. gonorrhoeae* was based on Gram stain morphology, oxidase test (Marion Scientific, Kansas City, Mo.), and carbohydrate fermentation with the Minitek System (BBL Microbiology Systems, Cockeysville, Md.) (20). To the vial containing the second swab, 1 ml of Gonozyme specimen dilution buffer was added; the tube was then vortexed, and the swab was discarded. A 200-μl portion of this eluent was placed into a well of a multiwell plate provided with the kit, and the non-antibody-treated bead was added to the well for adsorption of *N. gonorrhoeae*. The plate was incubated in a 37°C water bath for 15 min and then washed three times with distilled water. Rabbit antibody to *N. gonorrhoeae* (200 μl) was added to each well, and the plate was incubated and then washed again as above. Horseradish peroxidase-conjugated goat anti-rabbit antibody (200 μl) was then added, and the incubation-and-washing cycle was repeated. The beads were transferred to assay tubes, and 300 μl of o-phenyldiamine-2-hydrochloride was added. An orange color reaction is produced proportionate to the amount of enzyme attached to the bead. This reaction was stopped, after 10 min of incubation at room temperature, by the addition of 1 ml of 1 N HCl. The OD of the solution was read at 492 nm with a Quantum II spectrophotometer (Abbott Laboratories). One positive and three negative controls were assayed with each determination of EIA readings. The cutoff value for positive reactions was calculated as the mean of the three negative controls plus a factor of 0.19.

**Interpretation of results.** We compared Gram stain and EIA results with results of *N. gonorrhoeae*. Sensitivity, specificity, and positive and negative predictive values were calculated with formulas shown in the footnote of Table 1 (13, 28). Statistical analyses were performed with the Z proportionality test with a significance value of $P$ less than 0.05 (9).
Gonozyme consistently detected $\geq 10^4$ CFU of *N. gonorrhoeae* per ml at OD readings of greater than 0.6 (Fig. 1). The range of OD values at a concentration of $10^4$ CFU/ml probably also represented in part nonviable cells or cell debris since Gonozyme reactions are affected by the antigen concentration regardless of the number of viable cells.

Solutions which contained $\geq 10^6$ CFU/ml of all *Neisseria* which we tested had OD readings by EIA of 0.7 to 2.0 or greater. EIA reactions were also positive (OD > 2.0) with *Branhamella catarrhalis*, whereas *G. hemolysans* gave negative reactions. Solutions which contained $\geq 10^8$ CFU/ml of all the remaining aerobic bacteria which we tested had negative EIA reactions except for *Enterobacter aerogenes* and *Proteus vulgaris* (OD readings of 0.3 and 0.4, respectively). Solutions which contained *Bacteroides* sp. (except for *Bacteroides fragilis* ATCC 23745 and *Bacteroides thetaiotaomicron* ATCC 29742), *Peptostreptococcus anaerobius*, and *V. parvula* consistently yielded Gonozyme OD readings of 0.2 to 0.8. These positive results by EIA were eliminated by 1:1,000 dilutions of the bacterial suspensions.

In our clinical evaluation we studied specimens from 886 patients, including 295 males and 591 females. The sensitivities, specificities, and positive and negative predictive values of Gram stain and EIA in the three categories of patients are shown in Table 1. Overall, Gram stain and EIA were positive in 94 and 100, respectively, of 103 tests with males with positive cultures for *N. gonorrhoeae*. Likewise, of 111 females with positive cultures, Gram stain and EIA were positive in tests with 57 and 107 patients, respectively. The percentages of positive cultures for *N. gonorrhoeae* from males and females (34.9 and 18.8%, respectively) were comparable with those from other clinics for STD that treat large numbers of patients with gonorrhea (7, 14). The number of positive smear and culture results from patients who had positive EIA results are summarized in Fig. 2. For 61 females and 3 males, only the EIA test was positive. In contrast, for two females and one male the only positive result was a positive smear, whereas for three females and 2 males, only the cultures were positive.

We studied 1 male and 83 female contacts (group 1) to evaluate the usefulness of EIA in this group, but only the results for the women are shown in Table 1 because all tests were negative in the only male contact. The prevalence of positive cultures for *N. gonorrhoeae* in the 83 female contacts was higher than that in the other 468 females in group 3 (57.8 and 13.5%, respectively). In tests with female contacts, the sensitivities and specificities were similar to those

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**TABLE 1. Results of Gram stain, EIA, and culture for *N. gonorrhoeae* and data correlation of patients attending clinic for STD**

<table>
<thead>
<tr>
<th>Test group (n)</th>
<th>No. of patients with positive test</th>
<th>Sens$^a$</th>
<th>Spec$^a$</th>
<th>PPV$^c$</th>
<th>NPV$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contacts (group 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(females; n = 83)</td>
<td>GS*</td>
<td>25</td>
<td>52</td>
<td>48 (57.8)</td>
<td>50.0$^e$</td>
</tr>
<tr>
<td></td>
<td>EIA</td>
<td></td>
<td></td>
<td></td>
<td>97.9$^e$</td>
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<tr>
<td>Test of cure (group 2)$^g$</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Males (16)</td>
<td>GS</td>
<td>1</td>
<td>1</td>
<td>1 (6.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EIA</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Females (40)</td>
<td>GS</td>
<td>1</td>
<td>3</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EIA</td>
<td></td>
<td></td>
<td></td>
<td>92.5</td>
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<tr>
<td>Other patients (group 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males (278)</td>
<td>GS</td>
<td>97</td>
<td>105</td>
<td>102 (36.7)</td>
<td>91.2</td>
</tr>
<tr>
<td></td>
<td>EIA</td>
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<td>97.1</td>
</tr>
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<td>Females (468)</td>
<td>GS</td>
<td>37</td>
<td>117</td>
<td>63 (13.5)</td>
<td>52.4$^e$</td>
</tr>
<tr>
<td></td>
<td>EIA</td>
<td></td>
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<td>95.2$^e$</td>
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<tr>
<td>Total patients</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Males (295)</td>
<td>GS</td>
<td>98</td>
<td>106</td>
<td>103 (34.9)</td>
<td>91.3</td>
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<td></td>
<td></td>
<td>97.1</td>
</tr>
<tr>
<td>Females (591)</td>
<td>GS</td>
<td>63</td>
<td>172</td>
<td>111 (18.8)</td>
<td>51.4$^e$</td>
</tr>
<tr>
<td></td>
<td>EIA</td>
<td></td>
<td></td>
<td></td>
<td>96.4$^e$</td>
</tr>
</tbody>
</table>

$^a$ Sensitivity (%) = (no. positive by both test and culture/no. positive by culture) × 100.

$^b$ Specificity (%) = (no. negative by both tests and culture/no. negative by culture) × 100.

$^c$ PPV, Positive predictive value (%) = [true-positives/(true-positives + false-positives)] × 100.

$^d$ NPV, Negative predictive value (%) = [true-negatives/(true-negatives + false-negatives)] × 100.

$^e$ GS, Gram stain.

$^f$ EIA.

$^g$ P < 0.001 (Z proportionality test).

$^h$ Sensitivity and positive predictive value for Gram stain and EIA not indicated for group 2 because of the small number of positive results.
for tests with the remaining 468 females. However, the higher prevalence of positive cultures was reflected in the higher negative predictive value of EIA relative to Gram stain (Table 1).

Of the 56 patients who were evaluated for test of cure (group 2), only one male had a positive culture for *N. gonorrhoeae*. Thus, the specificities and negative predictive values of both tests were 100% for males. For the 40 females in this category, the specificities of Gram stain and EIA were 97.5 and 92.5%, respectively, and negative predictive values for both tests were 100% (Table 1). The specificities of EIA for females in groups 2 and 3 were comparable.

As noted above, of all 886 patients evaluated, the EIA of the exudates from 61 females and 3 males were positive, whereas smears and culture were negative for *N. gonorrhoeae*. In an attempt to explain this lower specificity of EIA for females, the OD readings of EIA from 172 females and 106 males with positive readings were plotted in relation to the results of smears and cultures that were positive for *N. gonorrhoeae* (Fig. 2). Of 61 females with positive Gonozyme only, 29 (48%) had OD readings of 0.2 to 0.4, whereas only 8 (7%) of 107 females with positive EIA and culture for *N. gonorrhoeae* had OD readings of 0.2 to 0.4. Eighty of 106 males and 37 (35%) of 107 females had both positive cultures for *N. gonorrhoeae* and OD readings of 2.0 or greater. A correlation of OD readings with Gram stain smears of the exudates of patients with positive cultures for *N. gonorrhoeae* disclosed that, although for most patients, high OD readings were obtained in cases in which smears disclosed many extra- and intracellular gram-negative diplococci, in some instances, few organisms were seen on smears from patients with high OD readings and vice versa.

**DISCUSSION**

Our clinical evaluation of EIA in 886 patients attending an STD clinic disclosed that Gonozyme EIA is a highly sensitive, rapid diagnostic method in males and females. In males, the sensitivity and specificity of EIA were comparable with those of Gram stain, whereas in females, EIA was more sensitive though less specific than Gram stain. These findings are similar to those of other published evaluations of Gonozyme (1, 6, 11, 22, 24, 26). It should be pointed out that at the time of our clinical study there was only one Gonozyme test available for evaluation. In the interim, a modified version (Gonozyme Diagnostic Kit; Abbott Laboratories) has been introduced for testing. The major difference from the earlier version is that the modified version requires 3 h to perform as compared with 1 h. We did preliminary in vitro testing of the new kit with serial dilutions of *N. gonorrhoeae* as outlined above and found that the OD values at concentrations of 10⁸ and 10⁹ CFU/ml were consistently >0.3, whereas at concentrations of 10⁵ CFU/ml or less, OD values were <0.4 (data not shown). Clinical studies are necessary to confirm the apparent increased sensitivity of this new EIA kit.

The high sensitivity of Gonozyme in our and other reported studies is in agreement with our in vitro data on OD readings of serial dilutions of *N. gonorrhoeae*. We found that Gonozyme detected gonococci at concentrations of 10⁵ CFU/ml or greater. Quantitative cultures of cervical secretions in females with gonococcal cervicitis have disclosed an *N. gonorrhoeae* concentration range of 10⁹ to 10⁵ CFU/ml (18). There are no comparable studies on concentrations of gonococci in male urethral swabs, although Aardoom et al. have estimated the growth density on urogenital swabs from a few patients with gonorrhea and found that males have a higher density of gonococci in their secretions than do females (1). A technical factor which may have decreased the sensitivity of EIA in our study was prior streaking of culture plates with the swab used for EIA, as noted by Schachter et al. (24).

We attempted to explain the lower specificity of EIA that we found in females. The OD readings of the exudates from swabs of cervical secretions in which only the EIA assay was positive were lower than those of swabs from patients both positive EIA and culture (Fig. 2). This may be partly due to false-negative cultures, since EIA will detect very low concentrations of *N. gonorrhoeae* as shown by our in vitro studies. A possible reason for false-negative cultures in females with cervical gonorrhea is inhibition of gonococci by vancomycin, which is included in the commonly used MTM and Martin-Lewis media (5, 10, 19, 23, 27, 30). The contribution of vancomycin-resistant strains of *N. gonorrhoeae* to possible false-negative cultures in our study is unclear, since we did not test the susceptibility to vancomycin and the auxotype pattern of our isolates. The predominantly black heterosexual characteristics of the patient population at the clinic would tend to minimize this factor, however, considering the decreased frequency of the arginine-, hypoxanthine-, and uracil-requiring auxotype of *N. gonorrhoeae* associated with vancomycin sensitivity among blacks (16).

Another possible explanation for positive EIA in females with negative cultures for *N. gonorrhoeae* is that these were false-positive reactions. Our in vitro data on OD readings of 0.2 to 0.8 by EIA when anaerobic bacteria were tested in high concentrations support the hypothesis that low positive OD readings by EIA in high-risk females with negative cultures for *N. gonorrhoeae* might be partly due to non-specific cross-reactions with anaerobes. We did not culture for anaerobes any of the cervical swabs with positive EIA results, but other studies have shown that anaerobes, including the ones we tested, can be found in high concentrations in healthy women (4).

The higher prevalence of gonorrhea among female contacts, which we and others (2, 15) have noted, is reflected in a much higher negative predictive value of EIA relative to
Gram stain (Table 1). Epidemiological treatment is practiced at some STD clinics for this high-risk group due to the low sensitivity of Gram stain and the risk of pelvic inflammatory disease and further transmission of gonorrhea in these individuals (15). Epidemiological treatment of contacts refers to the administration of antibiotics when gonorrhea is strongly suspected before the results of confirmatory cultures. Gonozyme offers an alternative to this type of treatment in females with suspected gonorrhea, due to the high sensitivity and high negative predictive value of this rapid test. Gonozyme results can be available on the day the patient visits the STD clinic. By administering treatment only to female contacts whose endocervical swabs are positive by Gonozyme, few females with gonorrhea would go untreated pending culture results. Moreover, patients without presumptive gonorrhea (negative EIA) would not receive antibiotics as predicted by epidemiological treatment.

We studied the Gonozyme test with patients evaluated at the STD clinic for test of cure after therapy for proven gonorrhea to determine whether residual nonviable gonococci in the urogenital tract would give positive EIA results and decrease the specificity of the test compared with culture. In our study, for 16 male and 40 female patients seen for test of cure at the clinic within 5 to 14 days of treatment, the specificities of Gonozyme were 100 and 92.5%, respectively. The specificity of Gonozyme in this subgroup of patients was higher than that for the remaining 278 males and 468 females in group 3 (96.6 and 85.9%, respectively). For all males and females studied by Aardoom et al. (1), the specificity of Gonozyme was 95.1%, whereas for patients examined after treatment, the specificity was 94.7%. Stamm et al. report that all 39 patients seen for test of cure 3 to 17 days after treatment had negative culture and EIA results (26). Based on these observations, we conclude that Gonozyme does not appear to detect residual nonviable gonococci on urogenital swabs from patients who had received treatment for gonorrhea 1 to 2 weeks earlier and are seen for test of cure.

In summary, Gonozyme is a highly sensitive, rapid method for detection of N. gonorrhoeae. It is comparable with Gram stain for males but much more sensitive than Gram stain for rapid diagnosis of gonorrhea in females. For female contacts, Gonozyme offers an alternative to epidemiological treatment. It may also be useful as a method for test of cure for patients treated for gonorrhea.

LITERATURE CITED