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Modified Enzyme Immunoassay for Detecting *Neisseria gonorrhoeae* Antigens

CHRISTOPHER J. PAPASIAN,[1,2] WILLIAM R. BARTHOLOMEW,[1,2,3] AND DANIEL AMSTERDAM[2,3]*

Department of Medical Technology, School of Health Related Professions,[1] and Department of Microbiology, School of Medicine,[3] State University of New York at Buffalo, and Erie County Laboratory,[2] Buffalo, New York 14215*

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A commercial modification of an enzyme immunoassay (EIA) (Gonozyme; Abbott Laboratories, North Chicago, Ill.) for detection of *Neisseria gonorrhoeae* antigens was compared with conventional culturing. Specimens from males and females were collected at a sexually transmitted disease clinic; additional female specimens were collected at an obstetrics and gynecology clinic. EIA sensitivity and specificity for males were 100 and 98.6%, respectively (68 negative, 34 positive, 1 false-positive, and 0 false-negative). EIA sensitivity and specificity for female sexually transmitted disease clinic patients were 74.4 and 95.7%, respectively (66 negative, 29 positive, 3 false-positive, and 10 false-negative) EIA sensitivity and specificity for obstetrics and gynecology clinic patients were 100 and 99.2%, respectively (6 positive, 119 negative, 1 false-positive, and 0 false-negative). In female patients from whom multiple swab specimens were collected, the sequence of specimen collection and subsequent EIA analysis affected sensitivity.

Gonorrhea ranks first among reported communicable diseases in the United States. Efforts to control its spread include screening asymptomatic females in obstetrics and gynecology (ObGyn) clinics and establishing federally subsidized clinics for the diagnosis, treatment, and epidemiological follow-up of individuals with sexually transmitted diseases (STD). Central to these efforts are the accuracy and time required for the laboratory diagnosis of gonococcal infections. Currently, the Centers for Disease Control recommends bacteriological culturing as the definitive method for diagnosing gonorrhea (2). Presumptive diagnosis of urogenital gonorrhea in males may be made on the basis of Gram staining, and this method is frequently used to identify individuals requiring antigonococcal therapy. With urogenital specimens from females, however, Gram staining is less sensitive than culturing, and its specificity varies with the expertise of the reader (4, 7). Bacteriological culturing is not an ideal diagnostic modality; it is labor intensive, requires technical competence, takes a minimum of 24 h, and is subject to insensitivity caused by loss of viability associated with improper specimen handling (10, 11).

An enzyme immunoassay (EIA) (Gonozyme; Abbott Laboratories, North Chicago, Ill.) for detecting antigens of *Neisseria gonorrhoeae* in urethral (male) or endocervical (female) specimens was recently marketed. It has been evaluated in several laboratories (1, 3, 6, 8, 9). In an earlier evaluation of this EIA, we found that the sensitivity and specificity exceeded 95% for specimens from males (6). For endocervical specimens, the sensitivity and specificity of the EIA were 79.2 and 87.2%, respectively (6). The manufacturer has made several modifications of the product, presumably to improve its validity for endocervical specimens. Our experience with this modified EIA product is presented in this report.

MATERIALS AND METHODS

Specimen collection. The patient population consisted of individuals who came to either the STD clinic at the Erie County Department of Health or the ObGyn clinic at the Erie County Medical Center for treatment. Specimens were collected from both asymptomatic and symptomatic individuals. A single endocervical swab specimen was collected from each ObGyn patient; it was cultured and then tested by EIA. Urethral (male) or endocervical (female) swab specimens were collected from each STD patient in duplicate; the first swab specimen was Gram stained and cultured, and the second swab specimen was cultured and then tested by EIA. This specimen collection protocol is in accordance with the manufacturer's recommendations. For many female patients seen at the STD clinic, the first endocervical swab specimen was also analyzed by EIA after Gram staining and culturing had been done. The culture and Gram stain results for the first swab specimens collected from STD clinic patients were not considered in this evaluation.

Bacteriological culturing. Isolation and identification procedures for *N. gonorrhoeae* were as previously described (6). If cultures were positive for *N. gonorrhoeae*, the number of colonies (CFU), up to 50, was enumerated. For STD clinic patients, CFU were enumerated from second swab specimen cultures only.

EIA specimen processing and assay procedure. Suspensions of antigenic material were prepared from swab specimens as described previously (6). The modified EIA was performed as directed by the manufacturer. The manufacturer's procedural modification entailed lengthening all four incubation periods; the first three were increased from 15 to 45 min each, and the fourth was changed from 10 to 30 min. Briefly, 0.2 ml of the controls or specimens from patients were incubated with treated beads (capable of binding *N. gonorrhoeae* antigens) in a 37°C water bath. Unbound material was aspirated, and the beads were washed with distilled water in the Pentawash system (Abbott). Anti-*N. gonorrhoeae* rabbit serum (0.2 ml) was added to each bead. The beads were incubated at 37°C and washed. Goat anti-rabbit immunoglobulin serum coupled with horseradish peroxidase (0.2 ml) was added to each bead and incubated at 37°C. Unbound material was aspirated, and the beads were washed. Each bead was submerged in 0.3 ml of freshly prepared *o*-phenylenediamine reagent and incubated at room

* Corresponding author.

temperature, and 1 ml of 1.0 M HCl was added to stop the reaction. The absorbance at 492 nm was determined with a Quantum (Abbott) photometric analyzer.

Interpretation of results. EIA results were interpreted as recommended by the manufacturer and as previously described (6). EIA was evaluated as a diagnostic test for gonorrhea by comparing EIA results with bacteriological culture results. EIA results were categorized as true-positive (TP; culture and EIA positive), true-negative (TN; culture and EIA negative), false-positive (FP; culture negative and EIA positive), and false-negative (FN; culture positive and 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; and negative predictive value = [TN/(FN + TN)] × 100.

Statistical analysis of EIA absorbance data. The mean absorbances ± standard deviations were determined by standard methods (5). The absorbances of the first and second swab specimens were compared by using Student's *t* test (5).

RESULTS

The EIA results for all three patient populations are shown in Table 1. For males, the sensitivity, specificity, and positive and negative predictive values all exceeded 97%. For ObGyn clinic patients, the sensitivity and specificity were 100 and 99.2%, respectively. Although the specificity of EIA was 95.7% for female STD clinic patients, the sensitivity was only 74.4%.

To determine whether sequential collection of endocervical swab specimens resulted in differential recovery of *N. gonorrhoeae* antigens, we compared the absorbances of the two swabs from 26 of the 29 female STD clinic patients with TP EIA results; paired swabs were not available from three patients. Of the 26 paired specimens, 8 were excluded because the quantity of antigen on each of the two swabs equaled or exceeded the amount required for a maximum absorbance. For the remaining 18 paired specimens, the mean absorbances were 1.3 (±0.700 SD) and 0.833 (±0.424 SD) for the first and second endocervical swab specimens, respectively. The difference in absorbances between the two swab specimens was statistically significant ($P < 0.025$), demonstrating that the first swab specimen contained a greater concentration of antigen than the second.

The number of CFU of *N. gonorrhoeae* was counted for culture-positive endocervical specimens (Table 2). Of the 45 culture-positive (TP plus FN) endocervical specimens, 32 had >50 CFU, and 12 had <50 CFU (1 was not counted). Of the 10 specimens yielding FN EIA results, 6 had <50 CFU, and 4 had >50 CFU. Of the 12 culture-positive specimens yielding <50 CFU, 6 (50%) produced a FN EIA result, whereas only 4 of the 32 (12.5%) specimens yielding >50 CFU generated a FN EIA result.

DISCUSSION

The sensitivity (100%) and specificity (98.6%) of EIA for male specimens would suggest that this method be considered as a suitable alternative to culturing for establishing definitive evidence of gonorrhea. For ObGyn clinic patients, EIA also performed well (sensitivity, 100%, specificity, 99.2%). A disadvantage of using EIA or other nonculture methods for diagnosing gonorrhea is that organisms are not available for assessing beta-lactamase production.

The specificity of EIA for specimens from female STD clinic patients was 95.7%. A chart review of the three female STD clinic patients whose specimens yielded FP EIA results revealed that two were asymptomatic and that one had a vaginal discharge of undetermined etiology. None of these three patients had received antibiotics for 1 week before cultures were done.

The sensitivity of EIA for specimens from female STD clinic patients was 74.4%. The sensitivity of a diagnostic test varies inversely with the number of specimens yielding FN results. As shown in Table 2, specimens yielding <50 CFU were more likely to produce a FN EIA result than specimens yielding >50 CFU. Clearly, the number of organisms recovered on a swab specimen is critical in determining the outcome of EIA. It is also apparent that bacteriological culturing for *N. gonorrhoeae* has a lower threshold for positivity (theoretically, one viable organism) than does EIA.

It appears that the study protocol, which maximized recovery of viable organisms for culturing and minimized recovery of antigen for EIA, biased results toward the insensitivity of EIA. The 10 specimens yielding FN EIA results were all collected from female STD clinic patients. A FN EIA result in these patients indicated that culturing of the second swab specimen yielded *N. gonorrhoeae* but that EIA of the same swab specimen was negative. Initially, no more than two procedures were performed on a single swab specimen. This decision stemmed from the manufacturer's impression that two procedures would remove a substantial quantity of antigenic material from the swab. As it is routine procedure at the Erie County Department of Health STD clinic to set up cultures and prepare films for Gram staining for all patients, two swab specimens had to be procured from each STD patient; the first swab specimen was used for culturing and Gram staining, and the second swab specimen was used for culturing and EIA. The first swab specimens from 5 of the 10 patients with FN EIA results were available for EIA. Of these five first swab specimens, three were positive by EIA, one was within the retest range (but was not retested), and one was negative by EIA. Thus, for four of these five patients with FN EIA results, the first swab specimen contained more antigenic material than the second. Similarly, for endocervical specimens yielding TP EIA results, first swab specimens had higher mean spectrophotometric absorbances than second swab specimens, once again

TABLE 1. EIA (Gonozyme) results for specimens collected from STD and ObGyn clinic patients

Patients	No. of specimens that were:				Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	TP	TN	FP	FN				
Males (STD clinic)	34	68	1	0	100	98.6	97.1	100
Females								
STD clinic	29	66	3	10	74.4	95.7	90.6	86.8
ObGyn clinic	6	119	1	0	100	99.2	85.7	100

TABLE 2. CFU versus EIA category for *N. gonorrhoeae* culture-positive endocervical specimens

CFU	No. of specimens tested	No. of specimens with:	
		TP EIA	FN EIA
<10	3	2	1
10–19	2	0	2
20–29	1	1	0
30–39	2	1	1
40–49	4	2	2
>50	32	28 (87.5)[a]	4 (12.5)[a]

[a] Numbers in parentheses are percentages.

indicating that second swab specimens contained less antigenic material than first swab specimens.

An additional factor probably contributing to the low sensitivity of EIA for females is that the second swab specimen was cultured before EIA analysis. This was done to alleviate the potential sampling error produced by comparing second swab specimen EIA results with first swab specimen culture results. Thus, as a result of our experimental protocol, antigenic material was removed from the endocervix (with the first swab) before a specimen was procured for EIA; additional antigenic material was then removed, by culturing, from this already deficient second swab specimen before it was tested by EIA. It is therefore reasonable to conclude that the specimen we tested by EIA would be more likely to give a FN EIA result than a routine clinical specimen which would not be subjected to substantial antigen removal. However, in clinical situations in which multiple endocervical swab specimens are procured and diagnostic tests for more than one etiological agent are performed, the order of specimen collection and subsequent EIA analysis can affect sensitivity. Apparently, the antigenic load of the infected male urethra is sufficient to overcome this biased experimental protocol, as the sensitivity of EIA for males was 100%.

This modified EIA is equal to or better than the previously marketed product for diagnosing gonorrhea in males; the sensitivity and specificity both exceeded 98%. Compared with a previously evaluated EIA (6), this product showed increased specificity for endocervical specimens. The sensitivity of the assay remained low for endocervical specimens collected at the STD clinic (74.4%); however, we believe that this value underestimates the true sensitivity of EIA.

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