COMPARISON OF MICROSCOPY, CULTURE AND ENZYME IMMUNOASSAY (GONOZYME®) FOR THE DETECTION OF *NEISSERIA GONORRHOEAE* IN UROGENITAL SPECIMENS

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SUMMARY. Urogenital specimens of male patients and female prostitutes were examined for gonorrhoea in a gonococcal antigen enzyme immunoassay (Gonozyne®), by microscopic examination of stained smears and by bacterial culture. Out of 18 male patients, 14 showed positive reactions (all 14 by Gonozyne and by microscopy, but only eight by culture also). The sensitivity and specificity of Gonozyne was 100% in reference to microscopy. The predictive value for a positive test and for a negative test was 100%. The sensitivity of Gonozyne in reference to culture was also 100%, but the specificity was only 40%, because of the low yield of positive cultures. The predictive value for a positive test was 57% and for a negative test 100%. Out of 189 female prostitutes, 41 (22%) had a positive reaction in at least one test (Gonozyne, microscopy and culture were positive in 10; Gonozyne and culture in three; Gonozyne and microscopy in 14; Gonozyne alone in 11; culture alone in three). The sensitivity of Gonozyne was 100% and specificity 92% in reference to microscopy. The predictive value for a positive test was 63% and for a negative test 100%. In reference to culture, the sensitivity was 81% and specificity 86%. The predictive value for a positive test was 34% and for a negative test 98%. In prostitutes, the rate of asymptomatic infections was 14%, if one assumed that all Gonozyne-positive results were truly positive. Gonozyne proved to be the most sensitive method for screening female patients. To discriminate possibly false positive reactions, Gonozyne-positive specimens should be corroborated, preferably by bacterial cultivation.

INTRODUCTION

From 1964 to the middle of the 1970s the incidence of gonorrhoea increased markedly all over the world; the incidence seems now to have levelled off (Catterall and Nicol, 1976; Skinner, Walker and Smith, 1977; Morello and Bohnhoff, 1980; Petzoldt, 1981). Reasons for this increase included greater promiscuity, decline of the use of the condom for contraception and a high rate of asymptomatic infections (Nielsen, Søndergaard and Ullman, 1975; Holmes, Eschenbach and Knapp, 1976; Handsfield, 1977). The increasing number of *Neisseria gonorrhoeae* strains resistant to penicillin

and to other β -lactam antibiotics (World Health Organization, 1977) may point to a new rise of gonococcal infections. Such dissemination may be impeded by the application of a rapid, reliable and practicable method for diagnosis.

Microscopy of stained smears is a rapid and generally sufficient method for the diagnosis in symptomatic males. In women, however, even if cultures are positive, c. 40–50% have negative smears (Danielsson, 1965; Danielsson and Johannisson, 1973; Morello and Bohnhoff, 1980).

Bacterial culture gives a delayed result. An enzyme immunoassay for the detection of gonococci might achieve the specificity and sensitivity of bacterial culture and the rapidity of diagnosis with stained smears (Sarafian and Young, 1982; Young *et al.*, 1983). For this purpose, we evaluated Gonozyne[®], an enzyme immunoassay for the detection of *N. gonorrhoeae* antigens developed by Abbott Laboratories (Max-Planck-Ring 2, D-6200 Wiesbaden-Delkenheim, FRG).

MATERIAL AND METHODS

Urogenital swabs of 207 patients (18 men and 189 female prostitutes) who consulted the Municipal Public Health Service, Aachen, were examined for gonococci by the following methods.

Microscopic examination. Smears were prepared at the bed-side, and stained with methylene blue and by Gram's method. They were considered positive if typical intracellular diplococci were found in the methylene-blue smear and gram-negative diplococci in the other smear.

Bacterial cultivation. The swabs, dispatched in a modified Stuart's transport medium (Temmler-Werke GmbH, Temmlerstrasse 2, 3550 Marburg/Lahn 1, FRG), were inoculated on a modified Thayer Martin medium (Sobczak, 1982) immediately after arrival, i.e., normally within 1–2 h, but sometimes 24–48 h after the specimens were collected. This reflects the usual transport time in practice. The agar plates were incubated at 36°C in CO₂ 5% for up to 3 days. The identity of suspected colonies was confirmed by a positive oxidase reaction, characteristic appearance in a gram-stained smear, carbohydrate degradation, immunofluorescence and specific coagglutination tests (Degner-Harms, Sobczak and Krause, in press).

Gonococcal antigen enzyme immunoassay (Gonozyne[®]). The swab for the Gonozyne test was dispatched together with the separate swab for bacterial cultivation. The test was done according to the instructions of Abbott Laboratories, and was briefly as follows. A specially treated bead was incubated with the swab specimen, to allow absorption of gonococcal antigens to the bead. With washing after each step, the bead was incubated next with rabbit anti-gonococcal antibody, then with a conjugate of goat anti-rabbit antibody and horseradish peroxidase, and finally with peroxidase-substrate to give a yellow-orange colour in a positive test.

RESULTS

Table I shows the findings with the three methods (Gonozyne, microscopy and culture). Out of 18 male patients, 14 showed a positive result (eight in all three tests, six in only Gonozyne and microscopy).

Out of 189 female patients, 41 (22%) had positive results: 10 with all three methods, three with only Gonozyne and culture, 14 with only Gonozyne and microscopy, 11 with only Gonozyne. For three other women, only culture showed a positive result; and in these, less than eight colonies of *N. gonorrhoeae* appeared after incubation for 72 h. In all cases where microscopy was positive, Gonozyne was also positive.

Of the 41 women with positive results, only 17 had clinical evidence of gonorrhoea; 10 were positive in all tests, six by Gonozyne and microscopy, one by Gonozyne and culture. Another four women had typical symptoms, but gave negative results in all

TABLE I
Detection of N. gonorrhoeae in urogenital specimens by Gonozyne, microscopy and bacterial culture

Results with			Number of specimens from			
Gonozyne	microscopy	culture	all patients (207)	men (18)	all women (189)	women with symptoms (21)
+	+	+	18	8	10	10
+	-	+	3	0	3	1
+	+	-	20	6	14	6
+	-	-	11	0	11	0
-	+	+	0	0	0	0
-	-	+	3	0	3	0
-	+	-	0	0	0	0
Positive in one or more tests			55	14	41	17
Negative in all tests			152	4	148	4

three tests. Thus 168 women had no clinical evidence of gonorrhoea, but 24 (14%) of them had positive tests.

Of the 14 male patients who had a positive result by one or more methods (table II), all were positive by Gonozyne and microscopy. Culture was positive only in 57%. Out of 41 prostitutes with positive results, 93% were positive by Gonozyne, 59% by microscopy, and 39% by culture. Considering that a positive test should be

TABLE II
Relative frequency of diagnosis of gonorrhoea by three methods

Method	Number (%) of positive results for		
	men (n = 14)*	women (n = 41)	all patients (n = 55)
Gonozyne	14 (100)	38 (93)	52 (95)
Microscopy	14 (100)	24 (59)	38 (69)
Culture	8 (57)	16 (39)	24 (44)

* n = Number of patients with at least one positive result.

TABLE III
Frequency with which a positive result by one method was corroborated by one or both other methods

Methods with positive results	Number (%) of positive results for		
	men (n = 14)*	women (n = 41)	all patients (n = 55)
Gonozyne and also microscopy or culture or both	14 (100)	27 (66)	41 (75)
Microscopy and also Gonozyne or culture or both	14 (100)	24 (59)	38 (69)
Culture and also Gonozyne or microscopy or both	8 (57)	13 (32)	21 (38)

* n = Number of patients with at least one positive result.

TABLE IV
Sensitivity, specificity and predictive values of three diagnostic methods*

Method I	Method II	Predictive value of							
		Sensitivity		Specificity		positive test		negative test	
		Men	Women	Men	Women	Men	Women	Men	Women
Gonozyne	Microscopy	100	100	100	92	100	63	100	100
Gonozyne	Culture	100	81	40	86	57	34	100	98
Microscopy	Culture	100	63	40	92	57	42	100	96

$$\text{Sensitivity} = \frac{\text{Number of results positive by both methods (I and II)}}{\text{Total number of results positive by method II}} \times 100\%.$$

$$\text{Specificity} = \frac{\text{Number of results negative by both methods (I and II)}}{\text{Total number of results negative by method II}} \times 100\%.$$

Predictive value of a positive test is defined as the percentage of method I-positive patients who were also positive by method II.

Predictive value of a negative test is defined as the percentage of method I-negative patients who were also negative by method II.

* Calculated from data in table I.

corroborated by another method (table III), Gonozyne was comparable with microscopy (both 100%) for diagnosis of gonorrhoea in males. In women, Gonozyne (66%) give slightly more positive results than microscopy (59%).

The sensitivity, specificity and predictive values of Gonozyne are presented in table IV. It is remarkable that, in reference to culture, Gonozyne was much more sensitive in female patients than microscopy (81% versus 63%). The specificity was slightly lower (86% versus 92%). The specificity in men was very low (40%), because of the relatively great number of negative cultures. In nearly all these cases the time between collection and inoculation of the specimens was > 24 h. The predictive value of a negative test with Gonozyne, indicating the probability that the patients were truly negative (Hofmann and Petzoldt, 1983) was for men 100% and for women $\geq 98\%$.

DISCUSSION

Urogenital specimens of 207 patients were examined for gonorrhoea by Gonozyne, microscopy and culture. Out of 18 male patients, all 14 with positive results had clinical signs and symptoms of gonorrhoea. In all these cases there was a 100% agreement between Gonozyne and microscopy. So, usually, in men with typical symptoms, microscopy is sufficient for diagnosis.

Of 168 women with no clinical evidence of gonorrhoea, 24(14%) had positive tests. This figure is low in comparison with values up to 80% mentioned by Holmes *et al.* (1976). Gonozyne detected 21 (88%), microscopy 8 (33%) and culture 5 (21%) of these asymptomatic cases. In 11 women without any clinical symptoms or signs, only Gonozyne showed a positive reaction (table I). It could not be clarified, whether these were asymptomatic infections or false positive reactions. In women, Gonozyne was positive in 93% as compared with 59% for microscopy. Moreover, Gonozyne results could be corroborated more often than other methods (table III).

In previous reports, c. 40–50% of women with positive cultures were negative by

microscopy (Danielsson, 1965; Danielsson and Johannisson, 1973; Morello and Bohnhoff, 1980; Young *et al.*, 1983). In our studies, out of 16 women with positive culture, 10 (63%) were positive by microscopy (table I). These results indicate that, in women, diagnosis of gonorrhoea by microscopy is unreliable. Gonozyme is much more sensitive than microscopy (81% versus 63%), with comparable specificities (table IV). These results are in agreement with the data of Hofmann and Petzoldt (1983).

The lowest yield of positive results was obtained by culture: 57% in males and 39% in females (table II). The viability of gonococci is drastically reduced by transport (Danielsson *et al.*, 1978), and only specimens inoculated at the bed-side give an acceptable rate of positive cultures. In optimal conditions, this is reported to be 80–90% (Schmale, Martin and Domescik, 1969). The advantage of Gonozyme is that it detects merely the antigens of *N. gonorrhoeae*; therefore specimens can be stored for 3–5 days before assay. The predictive value of a negative Gonozyme test is high ($\geq 98\%$), and this indicates its value as a screening test.

In conclusion, Gonozyme is a rapid method which can be easily performed. Results are available within 2 h. Killed gonococci in specimens are still detectable after 3–5 days. It seems to be useful as a screening test; in order to discriminate false positive reactions, Gonozyme-positive specimens should be corroborated by culture, preferably prepared at the bed-side. The other advantage of culture is the possibility of evaluating susceptibility to antibiotics.

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