J Clin Pathol 1983;**36**:674–677

Diagnosis of urogenital gonorrhoea by detecting gonococcal antigen with a solid phase enzyme immunoassay (Gonozyme™)

DAN DANIELSSON,* HARALD MOI,† LARS FORSLIN‡

*From the *Department of Clinical Microbiology and Immunology, the †Department of Dermato-Venereology and the ‡Department of Obstetrics and Gynaecology, Örebro Medical Center Hospital, S-701 85 Örebro, Sweden*

SUMMARY A solid phase enzyme immunoassay (Gonozyme™) was used to demonstrate gonococcal antigen in urogenital specimens. Urethral specimens from 101 men and cervical specimens from 150 women were examined, and the diagnostic yields were compared with those obtained by culture. The Gonozyme™ test was positive in 25 patients, 15 men and 10 women, and negative in 226 patients. Gonococci were isolated by culture in 23 of the patients, 12 men and 11 women. The Gonozyme™ test gave false-negative results in two men and one woman patient. The sensitivity of the test was 87% for the men and 91% for the women. Correspondingly, the test specificity was 94·3% for the men and 100% for the women, the predictive value of positive test 80% and 100%, and that of negative test 97·7 and 99·3% respectively. The Gonozyme™ test does not allow antibiotic sensitivity testings but has the advantage of rapidity and is not dependent upon viable organisms. The test is an attractive alternative to culture procedures for screening women patients with symptomatic or asymptomatic gonorrhoea.

A diagnosis of gonorrhoea cannot be made on clinical signs and symptoms only but must be based on the demonstration of gonococcal organisms in relevant specimens. Serological tests for demonstrating gonococcal antibodies in serum specimens have not proved to be sensitive and specific enough for a correct diagnosis because of inherent difficulties to differentiate a present infection from a previous one.[1] Hence, the demonstration of gonococci or gonococcal components in relevant specimens are at present the most reliable methods for the laboratory diagnosis of this disease.

Microscopy of smears, prepared from urogenital specimens and stained with methylene blue or Gram's method, is a rapid technique for a presumptive diagnosis with well recognised advantages and drawbacks.[2] Direct immunofluorescence,[3] radioimmunoassay,[4] limulus test[5] and genetic transformation[6] are other procedures that have been applied to identify gonococcal organisms or to indicate their presence. None of them, however, has superseded cultural procedures which, despite drawbacks such as labour, time and cost, are still the preferred method for the laboratory diagnosis of gonorrhoea.

A solid phase enzyme immunoassay (Gonozyme™) was recently developed by Abbot Laboratories (Chicago, Illinois, USA) for the rapid demonstration of gonococcal antigen in urogenital specimens of patients with gonorrhoea. In the present study the diagnostic yield of this technique was compared with that of culture.

Material and methods

PATIENTS

One hundred and one men and 150 women patients were included in the study. The patients were examined at the outpatient clinic for sexually transmitted diseases or at the outpatient clinic of the Department of Gynaecology of our Hospital because of a suspected genital infection.

DIAGNOSTIC PROCEDURES

Specimens were taken from the urethra of men and from the urethra, cervix, and rectum of the women

Accepted for publication 10 January 1983

with a cotton swab (ENT™ swab). These specimens were inoculated at the bedside on selective and non-selective gonococcal culture media.[7] The agar plates were then incubated at 36°C in 3–5% CO_2 atmosphere for two days, suspected colonies detected by the oxidase test and gonococci identified by coagglutination and fermentation tests.[8] All identified strains were tested for production of beta lactamase.[9] From some of the patients specimens were taken from the urethra of men and from the urethra and cervix of women for microscopy of smears stained in the usual way with methylene blue. Since this was not done regularly the findings were not included in the Results unless otherwise mentioned.

The same swabs from the urethra of the men and from the cervix of the women, which were used to inoculate the gonococcal culture medium, were inserted into tubes with 200 μl Gonozyme™ transport medium and transported to the laboratory for test with Gonozyme™ according to the instructions by the manufacturer. In this test treated beads are incubated with a swab specimen and appropriate controls. If the specimen contains *Neisseria gonorrhoeae,* the bacteria adhere to the bead. After aspiration of unbound material and washing of the bead, the bead is incubated with rabbit antigonococcal antibodies which react with gonococci on the bead. Next, the bead is incubated with horseradish peroxidase-labelled sheep antirabbit antibodies which react with the antigen-antibody complex on the bead. The presence of the enzyme on the bead surface is determined by incubating the washed bead with *o*-phenylenediamine (*o*PD) containing hydrogen peroxide. After incubation, a yellow-orange colour develops in proportion to the quantity of *N gonorrhoeae* antigens absorbed to the bead. The absorbances of controls and specimens are determined using a spectrophotometer with wavelength set at 492 nm. Specimens giving absorbance values equal to or greater than the cut-off value are considered positive for *N gonorrhoeae.* The whole test procedure including readings in the spectrophotometer takes approximately one hour.

Results obtained with culture were used as a reference to determine the sensitivity of the Gonozyme™ which was defined as the percentage of culture-positive patients who were Gonozyme™-positive. Specificity was defined as the percentage of culture-negative patients who were Gonozyme™-negative. The predictive value of a positive test was defined as the percentage of Gonozyme™-positive patients who were culture-positive. The predictive value of a negative test was defined as the percentage of Gonozyme™-negative patients who were culture-negative.

Table 1 *Comparison of the results obtained with culture and Gonozyme™ in women*

Gonozyme™	*Culture*		*Total*
	Positive	*Negative*	
Positive	10	0	10
Negative	1	139	140
Total	11	139	150

Table 2 *Comparison of the results obtained with culture and Gonozyme™ in men*

Gonozyme™	*Culture*		*Total*
	Positive	*Negative*	
Positive	10	5	15
Negative	2	84	86
Total	12	89	101

Results

The results are summarised in Tables 1 and 2. Gonococci were detected by culture procedures in 12 men and 11 women, and by Gonozyme™ in 15 men and 10 women. Amongst the women, the Gonozyme™ and culture results agreed in all except one case with a positive culture but negative Gonozyme™. No information was available regarding microscopy of a smear from this patient. Two men were positive by culture but negative by Gonozyme™. One of these men had asymptomatic gonorrhoea and was negative in direct microscopy. Five men were positive in Gonozyme™ but negative by culture. Two of these men were contacts to women with gonorrhoea and on microscopy of methylene blue stained smears were considered positive. In the other three men no evidence of positive epidemiology was obtained and methylene blue stained smears were considered negative.

Statistical methods were applied to analyse the results with Gonozyme™ by using the results with culture as reference. For both men and women, the Gonozyme™ showed a sensitivity of 87%, a specificity of 97·8%, a predictive value of positive test of 80%, and a predictive value of negative test of 98·7%. The corresponding figures for the men were 83%, 94·3%, 66·7% and 97·7% and for the women 90·9%, 100%, 100% and 99·3%, respectively.

Discussion

We previously showed that the highest isolation rates of gonococci were obtained when the urogenital specimens were inoculated at the bedside with the use of both selective and non-selective culture

media.[7] In the present study this procedure was chosen to determine the accuracy and efficiency of the Gonozyme™ test which is a solid phase enzyme immunoassay for the demonstration of gonococcal antigens in urogenital specimens. Compared with culture of 250 patients the overall sensitivity of the test was found to be 87%, the specificity 97·3%, the predictive value of a positive test 80%, and that of a negative test 98·7%.

It was of interest to note that the accuracy and efficiency of the Gonozyme™ test, as compared with culture, was higher in the women patients than in the men. Only one false-negative test was obtained in the former group, and accordingly, the sensitivity was 91%, the specificity and predictive value of a positive test 100%, and the predictive value of a negative test 99·3%.

The sensitivity of the Gonozyme™ test was lower in the men and false-negative results were obtained in two patients. This might reflect too low a concentration of gonococcal antigen to be detected in the specimens of these two patients, and could be due to the fact that the swabs used for the Gonozyme™ test were first streaked on the agar plates (see Material and methods). Five of the men were positive by the Gonozyme™ test but negative by culture which could then be considered as false-positive results. However, two of these men had a positive epidemiology for gonorrhoea (their women contacts had gonorrhoea), and the urethral smears were considered positive by direct microscopy. For the other three men there was no indirect evidence of gonorrhoea. If corrections are made for this in the men, the sensitivity of the test would be 85·7%, the specificity 96·6%, the predictive value of positive test 80% and that of negative test 97·7%. The reasons for the discrepancies between culture and the Gonozyme™ for three of the men are not clear, and at present it cannot be decided whether it is due to non-specific reactions or a high sensitivity of the test.

Several factors must be considered in the cultural procedures of gonococci since they are known to be fastidious organisms that are sensitive to various kinds of physicochemical factors. The importance of using highly enriched media to grow gonococci cannot be overemphasised. Many physicians in the Scandinavian countries have to send specimens to a diagnostic laboratory, and usually the specimens are transported in modified Stuart's transport medium.[10] Culture after transport will, however, give a lower diagnostic yield than culture performed at the bedside. The yield is drastically reduced when the specimens are transported for two days or more.[7] From this point of view the Gonozyme™ offers an advantage since it is not dependent upon viable organisms.

Rapidity is another advantage of the Gonozyme™ test and a diagnosis may be made within one hour of the specimen arriving at the laboratory. This results in a minimum of delay in the decision to initiate therapy. Microscopy of stained smears gives the same advantages and this technique, when performed by an experienced examiner, is at least as sensitive as culture or the Gonozyme™ test in men with acute gonorrhoea. The situation is quite different among women and it has been shown that 40–80% of those who are culture-positive are missed by direct microscopy.[11,12] In the present study the Gonozyme™ test had an accuracy and efficiency well comparable with culture, although it took two, three or more days to give a specific diagnosis. On the other hand, culture allows sensitivity testings which is important with regard to PPNG strains. From this point of view it is desirable that the Gonozyme™-positive specimens are cultured to prove or disprove the presence of PPNG which might be important in those areas with a high prevalence of such strains.

In its present status the Gonozyme™ test is only intended for urogenital specimens, which is a drawback if extragenital specimens, for example oropharyngeal ones, are examined. Regardless of these drawbacks the Gonozyme™ test seems to have a great potential use for screening women patients for gonorrhoea, especially those who are considered as high risk patients and those who are asymptomatic. The present study indicates that it is sufficient to test the cervical specimens of women patients and this is also economically attractive.

References

[1] Holmes KK, Buchanan TM, Adam JL, Eschenbach DA. Is serology useful in gonorrhoea? A critical analysis of factors influencing serodiagnosis. In: *Immunobiology of Neisseria gonorrhoeae*. American Society for Microbiology DC, 1978: 370–6.

[2] Reyn A. Laboratory diagnosis of gonococcal infections. *Bull WHO* 1965;**32**:449–69.

[3] Danielsson D, Forsum U. Diagnosis of Neisseria infections by defined immunofluorescence. Methodologic aspects and applications. *Ann N Y Acad Sci* 1975;**254**:334–49.

[4] Thornley MJ, Wilson DV, Demarcho de Hormaeche R, Oates JK, Coombs RRA. Detection of gonococcal antigens in urine by radioimmunoassay. *J Med Microbiol* 1978;**12**:161–75.

[5] Spagna VA, Prior RB, Perkins RL. Rapid presumptive diagnosis of gonococcal urethritis in men by the limulus lysate test. *Br J Vener Dis* 1979;**55**:179–82.

[6] Janik A, Juni E, Heym GA. Genetic transformation as a tool for detection of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1976;**4**:71–81.

[7] Danielsson D, Sandström E, Kjellander J, Moi H, Wallmark G. Culture diagnosis of gonorrhoea—a comparison between two standard laboratory methods and a commercial gonococcal culture kit (Kvadricult®). *Acta Derm Venereol (Stockh)* 1978;**58**:69–73.

[8] Olcén P, Danielsson D, Kjellander J. Laboratory identification of pathogenic Neisseria with special regard to atypical strains: an evaluation of sugar degradation, immunofluorescence and co-agglutination test. *Acta Pathol Microbiol Scand* [*B*] 1978;**86**:327–34.

[9] O'Callaghan CH, Morris A, Kirby SM, Shingler AH. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother* 1972;**1**:283–7.

[10] Gästrin B, Kallings LO. Improved methods for gonococcal sampling and examination on a large scale. *Acta Pathol Microbiol Scand* 1968;**74**:362–70.

[11] Danielsson D. The demonstration of *N gonorrhoeae* with the aid of fluorescent antibodies. 6. A comparison of conventional methods and fluorescent antibody (FA) techniques with counterstaining for the demonstration of *N gonorrhoeae*. *Acta Derm Venereol (Stockh)* 1965;**45**:74–80.

[12] Danielsson D, Johannisson G. Culture diagnosis of gonorrhoea. A comparison of the yield with selective and non-selective gonococcal culture media inoculated in the clinic and after transport of specimens. *Acta Derm Venereol (Stockh)* 1973;**53**:75–80.

Requests for reprints to: Dr D Danielsson, Department of Clinical Microbiology and Immunology, Örebro Medical Center Hospital, S-701 85 Örebro, Sweden.