Identification and Penicillinase Testing of *Neisseria gonorrhoeae* from Primary Isolation Cultures on Modified New York City Medium

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The fluorescent-antibody test, rapid carbohydrate utilization test (RCUT), and a test for penicillinase production were performed on the bacterial growth from primary cultures on modified New York City medium. Of 134 gonococcal infections in men, 88.8% were diagnosed by the fluorescent-antibody test and 70.9% by the RCUT after incubation for 24 h; the corresponding figures for 75 infections in women were 86.7 and 54.7%, respectively. After incubation for 48 h, 100% of infections were diagnosed by the fluorescent-antibody test, whereas 88.8% of infected males and 86.7% of females were also diagnosed by the RCUT. Primary isolation cultures on modified New York City medium proved suitable for determining the ability of strains to produce penicillinase by a modified RCUT procedure. The method of isolation and identification by using primary cultures from modified New York City medium is both rapid and economical. The rapidity of diagnosis provided by the RCUT makes this a very useful diagnostic method, particularly in laboratories lacking immunofluorescence equipment. Since the penicillinase production test forms part of a routine identification procedure, no extra culture media or subcultures are required. The rapidity of this test should make it of value in tracing contacts of patients infected with penicillinase-producing strains.

The rapid cultural diagnosis of gonorrhea not only benefits the individual patient but also aids control of the infection within the community (4). The time taken for diagnosis by conventional biochemical methods can be substantially reduced by using a combination of the delayed fluorescent-antibody (FA) method and a rapid carbohydrate utilization test (RCUT) (6, 10). Unfortunately, slow growth of gonococci on Thayer-Martin (TM) medium and the need to subculture isolates prior to performing the RCUT (10) limit the value of these rapid techniques. To improve further the speed of cultural diagnosis, we have modified the New York City medium described by Faur et al. (2, 3), enabling us to carry out the RCUT directly from the primary isolation plate. In an earlier trial (9), modified New York City (MNYC) medium gave a significantly higher rate of initial recovery of *Neisseria gonorrhoeae* than TM medium.

The recent emergence of penicillinase-producing strains of *N. gonorrhoeae* highlights the importance of laboratory surveillance of the penicillin sensitivity of clinical isolates (7). Penicillinase-producing gonococci can be detected simply and economically by a modification of the RCUT (8). When MNYC medium is used for

primary isolation, penicillinase testing can be performed directly from the isolation plate. This paper reports our experience using MNYC medium for the rapid culture, identification, and penicillinase testing of *N. gonorrhoeae* in a routine diagnostic laboratory.

MATERIALS AND METHODS

Isolation medium. MNYC medium contained Difco GC base enriched with 10% (vol/vol) human blood lysed with 0.5% (wt/vol) saponin, 2.5% (vol/vol) yeast dialysate prepared as described by Faur et al. (3), 0.1% (wt/vol) glucose, lincomycin (1.0 µg/ml), colistin (6 µg/ml), amphotericin B (1.0 µg/ml), and trimethoprim lactate (6.5 µg/ml).

Specimens. Cultures were obtained from patients attending the Department of Venerology, The Royal Infirmary of Edinburgh, during April, May, and June 1977. Urethral cultures were taken routinely in all male patients, and rectal and pharyngeal cultures were also taken as a routine from homosexual men. In female patients, urethral, cervical, rectal, and, if indicated, pharyngeal cultures were made. Material was inoculated directly onto culture plates at the time of the patient examination.

Culture and examination of plates. After inoculation, plates were held at 36°C in a carbon dioxide-enriched (10%) atmosphere. After incubation for 24 h plates were examined, any suspect colonies were tested

for the oxidase reaction, and Gram smears were prepared. Presumptive gonococci (oxidase-positive gram-negative diplococci) were then identified fully and tested for penicillinase production as outlined below. Negative cultures were reexamined after incubation for 48 h. If presumptive gonococci were present, they were examined by the FA technique, and a subculture was made onto a fresh MNYC plate for carbohydrate and penicillinase tests next day.

FA test. The FA test was performed using Difco fluorescein-labeled anti-gonococcal conjugate as described previously (10). Each day, stock strains of *N. gonorrhoeae* and *N. meningitidis* were used as positive and negative controls, respectively.

RCUT and penicillinase test. The test for penicillinase production (8) depends on the color change of a pH indicator when acid is produced from the splitting of ampicillin to penicilloic acid. Sodium ampicillin (Beecham Research Laboratories) was dissolved in buffer-salt solution (BSS) (5.2 mmol of phosphate buffer per liter containing 0.8% [wt/vol] potassium chloride and 0.01% phenol red, pH 7.10 to 7.15) to a concentration of 250 mg/ml; 2 ml of the solution was dispensed in 100- μ l volumes and stored at -20°C for up to 2 weeks.

Five tubes (70 by 10 mm) were used for each gonococcal isolate tested; 20 μ l of 10% aqueous (wt/vol) glucose, maltose, and sucrose and 20 μ l of ampicillin (250 mg/ml in BSS) were added to four individual tubes, followed by 100 μ l of BSS. If there was sufficient growth of presumptive gonococci on the primary isolation plate at 24 h, this was harvested using a cotton-tipped swab, and a thick suspension was made in 300 μ l of BSS in the fifth tube and mixed well; 30 μ l of this suspension was transferred to each sugar-containing tube and to the ampicillin tube. Tubes were shaken and incubated at 37°C in a water bath. A positive result was normally available in 30 to 60 min and a definitive reading at 3 h. A yellow color (occasionally yellow-orange) is positive; red is negative. Each day, a penicillinase-positive and penicillinase-negative strain of *N. gonorrhoeae* and a strain of *N. meningitidis* were set up as controls.

If there was insufficient growth of presumptive gonococci on the primary isolation plate at 24 h, a subculture was made onto MNYC medium, and the RCUT and penicillinase tests were carried out after overnight incubation.

RESULTS

During the period of the study, excluding specimens requiring processing over the weekend period, there were 134 cases of gonorrhea in men and 75 cases in women. All isolates of *N. gonorrhoeae* produced acid from glucose only, were FA-test positive, and did not produce penicillinase. Tables 1 and 2 show the time taken to obtain these results in male and female patients, respectively.

From Tables 1 and 2 it is seen that the FA test was equally efficient in the rapid diagnosis of gonorrhea in men (88.8% of patients diagnosed at 24 h) and in women (86.7% in 24 h). In

contrast, rapid diagnosis by the RCUT was more suitable for isolates from men than from women (70.9% and 54.7% of patients diagnosed at 24 h, respectively). After subculture and incubation of any suspect gonococci present at 24 h, there was little difference in the percentage of men and women diagnosed at 48 h by the RCUT (88.8 and 86.7% of patients, respectively). Tests for penicillinase production were always performed at the same time as the RCUT.

To determine whether differences exist in the rapidity of diagnosis for particular culture sites, the number of urethral, cervical, rectal, and fauces cultures that could be tested at 24 h was determined and is shown in Tables 3 and 4.

There was little difference in rapidity of diagnosis by the FA test in various culture sites in both male and female patients. The FA test was of particular value in the rapid identification of gonococci in rectal cultures. Positive rectal cultures in women generally produced a more rapid and abundant growth of gonococci with less growth of unwanted microorganisms than did rectal cultures in men; 96.0% of positive rectal cultures in women were positive by the FA test at 24 h, compared with only 81.8% of positive cultures in men, and the corresponding figures for the RCUT at 24 h were 68.0 and 45.5%, respectively.

DISCUSSION

Many workers have emphasized the importance of rapid cultural diagnosis of gonorrhea (1, 4, 5). Our results with MNYC medium show that almost 90% of infections were diagnosed by immunofluorescence at 24 h. In a previous report

TABLE 1. Time taken to establish diagnosis in 134 cases of gonorrhea in men

Test	No. of patients (%) at ^a :		
	24 h	48 h	72 h
FA	119 (88.8)	15 (11.2)	0 (0)
RCUT	95 (70.9)	24 (17.9)	15 (11.2)
Penicillinase production	95 (70.9)	24 (17.9)	15 (11.2)

^a One or more isolates tested per patient.

TABLE 2. Time taken to establish diagnosis in 75 cases of gonorrhea in women

Test	No. of patients (%) at ^a :		
	24 h	48 h	74 h
FA	65 (86.7)	10 (13.3)	0 (0)
RCUT	41 (54.7)	24 (32.0)	10 (13.3)
Penicillinase production	41 (54.7)	24 (32.0)	10 (13.3)

^a One or more isolates tested per patient.

TABLE 3. *Rapidity of diagnosis for different culture sites in male patients*

Site (no. positive ^a)	No. of isolates (%) at 24 h by:	
	FA test	RCUT and penicillinase production test
Urethra (122)	109 (89.3)	89 (72.9)
Rectal (11)	9 (81.8)	5 (45.5)
Fauces (5)	5 (100.0)	3 (60.0)
Total (138)	123 (89.1)	97 (70.3)

^a Includes those positive at 24 and 48 h.

TABLE 4. *Rapidity of diagnosis for different culture sites in female patients*

Site (no. positive)	No. of isolates (%) at 24 h by:	
	FA test	RCUT and penicillinase production test
Urethra (47)	41 (87.2)	34 (72.3)
Cervix (66)	57 (86.4)	41 (62.1)
Rectal (25)	24 (96.0)	17 (68.0)
Fauces (4)	3 (75.0)	2 (50.0)
Total (142)	125 (88.0)	94 (66.2)

^a Includes those positive at 24 and 48 h.

(10) using TM medium, only 60% of infections were diagnosed at 24 h by immunofluorescence. Growth on TM medium was unsuitable for carrying out the RCUT, making subculture necessary before final identification (10).

We also found that the original NYC medium, which contains 0.5% (wt/vol) glucose (3), was unsuitable for the RCUT due to excess acid production by the bacteria during overnight culture. To enable the RCUT to be performed directly from the primary isolation plate, MNYC medium was supplemented with only 0.1% (wt/vol) glucose.

By carrying out the RCUT directly from the primary isolation plate, a diagnosis could be made in 55% of infected women and 71% of infected men after 24 h of incubation. This difference, which is statistically significant ($0.05 > P > 0.02$), could result from more frequent and/or abundant growth of unwanted microorganisms on the primary cultures from women and/or from certain cultures having little gonococcal growth present. Direct observation favors the latter explanation; cultures from women were rarely heavily contaminated, but occasionally cervical cultures produced a less abundant growth of gonococci. Usually these cultures were from patients in whom the cervical culture was the only one positive.

Although FA testing alone is normally considered sufficient identification in the case of anogenital isolates, carbohydrate utilization tests

are usually performed in the case of isolates from oral, disseminated, and conjunctival infections. Carbohydrate utilization tests are necessary to identify neisseriae giving negative or equivocal results in the FA test. The rapidity of diagnosis provided by the application of the RCUT to the MNYC isolation plate makes this a very useful diagnostic method in all laboratories but particularly in those lacking immunofluorescence facilities. To ensure reliable results, only overnight cultures should be used for the RCUT.

Primary isolation cultures on MNYC medium proved suitable for testing for penicillinase production. Since this test (8) forms part of a routine identification procedure, no extra culture media or subcultures are required, making it a simple, inexpensive, and very rapid method of testing for penicillinase production. Although none of the gonococcal strains in this study produced penicillinase, 2 months after the study was concluded a penicillinase-producing strain was detected. The test was positive after 24 h of incubation and 1 day before disk sensitivity results were known. Such a saving in time should allow rapid tracing of contacts and could make an important contribution to the control of penicillinase-producing strains.

We conclude that the above scheme of isolation, identification, and penicillinase-production testing using primary cultures from MNYC medium is rapid and economical in terms of culture media and time required for examination of plates and performance of tests. It may be particularly useful in developing countries.

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