

Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium

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SUMMARY A simply prepared modified New York City medium, designated MNYC was compared with Thayer Martin (TM) medium for the cultural diagnosis of gonorrhoea. MNYC medium contained lincomycin, commercial gonococcal base and lysed whole blood, whereas the original New York City medium contained fresh horse plasma and haemoglobin solution, a basal medium prepared from basic ingredients and vancomycin. Using MNYC medium gonococci were cultured from 96.1% of men and 100% of women with gonorrhoea (positive film and/or culture) compared with only 77.6% and 69% respectively using TM medium. There were no patients positive by culture on TM medium but negative by culture on MNYC medium. The proportion of men with positive films but negative culture was reduced from 17.1% on TM medium to 3.9% on MNYC medium. There were no women with positive films but negative cultures on MNYC medium compared with 19% on TM medium. MNYC medium is recommended as a simply prepared and highly efficient medium for the cultural diagnosis of gonorrhoea.

Introduction

Although microscopical examination is important in making a presumptive diagnosis of gonorrhoea so allowing immediate treatment, cultures are obligatory in the diagnosis of rectal, oral, disseminated, and asymptomatic infections in both sexes. They are also essential in determining antibiotic sensitivities and for evaluating treatment.

The selective medium of Thayer and Martin (1966) is widely used in many laboratories and has increased the number of positive isolates from all sites, but it has proved particularly valuable in isolating the gonococcus from heavily contaminated sites such as the rectum (Roepstorff and Hammarström, 1966). Unfortunately, at least 3% of gonococcal strains are sensitive to vancomycin at a concentration of 3 µg/ml (Reyn, 1969) and are therefore unlikely to be detected when Thayer Martin (TM) medium, containing vancomycin 5 µg/ml, is used. Also, isolates tend to grow slowly on TM medium and produce small colonies.

On introducing delayed immunofluorescence for the routine identification of gonococci we found that

slow growth on TM medium became the limiting factor in the speed of cultural diagnosis. Faur *et al.* (1973a, b) described a new selective medium, designated NYC (New York City) medium, which provided luxuriant growth of pathogenic neisseriae after incubation for 24 hours. NYC medium essentially consists of a proteose peptone-corn starch agar buffered base to which is added a haemoglobin solution prepared from fresh horse erythrocytes, with horse plasma, yeast dialysate, glucose and the antibiotics—vancomycin, colistin, amphotericin B, and trimethoprim lactate.

The aim of this investigation was to compare culture results obtained using our standard TM medium with those obtained using a simply prepared modification of NYC medium.

Material and methods

MEDIA

TM medium comprised lab m Columbia agar base (London Analytical and Bacteriology Media Ltd, 50 Mark Lane, London) supplemented with 10% (by vol.) heated (56°C for 60 min) human blood and the antibiotics vancomycin (4 µg/ml), colistin (6 µg/ml), and nystatin (10 µg/ml).

MNYC (Modified New York City) medium A comprised Difco gonococcal base enriched with

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10% (by vol.) defibrinated horse blood (Wellcome Ltd) lysed with 0.5% (by vol.) saponin, 2.5% (by vol.) yeast dialysate prepared as described by Faur *et al.* (1973a), 0.1% (by vol.) glucose, lincomycin (0.5 µg/ml), colistin (6 µg/ml), amphotericin B (1.0 µg/ml), and trimethoprim lactate (6.5 µg/ml).

MNYC medium B was prepared as above but with lincomycin at a concentration of 1.0 µg/ml.

MNYC medium C was prepared as medium B but contained 10% (by vol.) human blood, lysed with 0.5% (by vol.) saponin, in place of defibrinated horse blood.

In the first part of the trial 'split' plates were made by pouring 10 ml of MNYC medium A into one half and 10 ml of TM medium into the other half of diametrically partitioned and vented Petri plates.

In the second part of the trial split plates contained MNYC medium B and MNYC medium C.

Determination of minimum inhibitory concentration (MIC) to vancomycin and lincomycin

At the time of isolation, a suspension of each gonococcal isolate was made in skimmed milk (10%), quick frozen with a mixture of solid carbon dioxide and acetone and stored at -20°C. Later, strains were recovered and the MICs to vancomycin and lincomycin determined using sensitivity test agar (Oxoid Ltd) supplemented with 10% lysed human blood: vancomycin and lincomycin were added at concentrations of 2, 4, 8, 16, and 32 µg/ml.

PATIENTS

The patients in this study attended the Department of Venereology, Edinburgh Royal Infirmary, between 15 November 1976 and 21 February 1977.

In men, if a urethral discharge was present, or if there was presumptive evidence of contact with gonorrhoea, a Gram-stained smear of urethral discharge was examined microscopically and material inoculated directly on to culture plates as described below. In the case of homosexuals, material from the urethra, rectum and pharynx was cultured routinely, and, if negative on the first occasion, cultures were repeated twice at weekly intervals.

In women, Gram-stained smears of material from the urethra and cervix, and cultures from the urethra, cervix, rectum and, if indicated, from the pharynx, were taken. If the first cultures for *N. gonorrhoeae* were negative, these were generally repeated twice at weekly intervals.

In the first part of the study, 15 November until 31 December 1976, all specimens for *N. gonorrhoeae*, including tests of cure when required, were plated directly on to both TM medium and MNYC medium A: the order of inoculation of the medium alternated with each patient. During this period

there were 256 women yielding 2124 cultures and 422 men yielding 600 cultures.

In the second part of the study, 13 January until 21 February 1977, when cultures were made on MNYC medium B and MNYC medium C there were 287 women and 417 men.

INCUBATION AND IDENTIFICATION

After inoculation, plates were held at 36°C and transferred to the laboratory within a few hours where they were incubated at 36°C in a carbon dioxide enriched (10%) atmosphere. After incubation for 24 hours, plates were examined and any suspect colonies tested for the oxidase reaction and Gram smears prepared. Oxidase positive Gram-negative diplococci were identified by the rapid carbohydrate utilisation test and by delayed immunofluorescence with Difco fluorescein-labelled anti-gonococcal conjugate as described by Young *et al.* (1976): all isolates of *N. gonorrhoeae* produced acid from glucose only and gave a positive fluorescent antibody test. Negative cultures were re-examined after incubation for 48 hours.

Results

STUDIES WITH MNYC MEDIUM A

This medium contained 10% horse blood and lincomycin (0.5 µg/ml).

FEMALE PATIENTS

The numbers of specimens positive for *N. gonorrhoeae* by culture on TM medium are compared with those on MNYC medium A in Table 1.

Table 1 Results of culture for *Neisseria gonorrhoeae* when 2124 specimens from 256 female patients were plated on TM medium and MNYC medium A

Site	No. sampled	No. of cultures positive	
		On TM medium	On MNYC medium A
Urethra	683	16	29
Cervix	681	29	40
Rectum	586	5	8
Throat	160	2	2
Other	14*	4†	7
Total	2124	56	86

*Bartholin's gland (11), high vaginal swab (1), wrist pustule (1), coil (1)

†All Bartholin's gland

The 56 cultures positive on TM medium corresponded to 29 patients while the 86 cultures positive on MNYC medium A corresponded to 42 patients. Results of culture and microscopical examination for these patients are shown in Table 2.

Table 2 *Results of microscopical examination and culture on TM medium and MNYC medium A for 42 female patients with gonorrhoea*

Pattern of results	Patients with gonorrhoea and pattern of results			
	On TM medium		On MNYC medium	
	No.	(%)	No.	(%)
Culture + smear +	20	(47.6)	28	(66.7)
Culture + smear -	6	(14.3)	9	(21.4)
Culture + smear 0	3	(7.1)	5	(11.9)
Culture - smear +	8	(19.0)	0	(0.0)
Total	37	(88.0)	42	(100)

+ = Positive, - = negative, 0 = no smear

Of the 13 patients with gonorrhoea detected by culture on MNYC medium A but missed by culture on TM medium, eight were smear positive, three were smear negative, and there were no corresponding smears for two patients. Smears were positive but TM cultures negative in 19.0% (8/42) of patients with gonorrhoea. There were no positive smears with negative cultures when MNYC medium A was used. Microscopical examination detected 66.7% (28/42) of the women with gonorrhoea. Excluding the five patients from whom no smears were available, microscopical examination detected 75.7% (28/37) of infected patients. A combination of microscopy and culture on TM medium detected 88% (37/42) of the positive patients whereas culture on TM medium alone detected only 69.0% (29/42).

MALE PATIENTS

The numbers of specimens positive for *N. gonorrhoeae* by culture on TM medium are compared with those on MNYC medium A in Table 3.

Table 3 *Results of culture for Neisseria gonorrhoeae when 600 specimens from 422 male patients were plated on TM medium and MNYC medium A*

Site	No. sampled	No. of cultures positive	
		On TM medium	On MNYC medium A
Urethra	394	53	69
Rectum	43	3	4
Throat	152	0	0
Other	11*	0	0
Total	600	56	73

*Prostatic secretion (3), urine thread or deposit (7), eye swab (1)

As only one culture site was positive for each patient there were 56 patients with gonorrhoea detected by TM culture and 73 patients detected by culture on MNYC medium A. Results of culture and microscopical examination for these patients

and three patients from whom positive smears but negative cultures were obtained are shown in Table 4.

Table 4 *Results of microscopical examination and culture on TM medium and MNYC medium A for 76 male patients with gonorrhoea*

Pattern of results	Patients with gonorrhoea and pattern of results			
	On TM medium		On MNYC medium	
	No.	(%)	No.	(%)
Culture + smear +	40	(52.6)	53	(69.7)
Culture + smear -	8	(10.5)	10	(13.2)
Culture + smear 0	8	(10.5)	10	(13.2)
Culture - smear +	13	(17.1)	3	(3.9)
Total	69	(90.8)	76	(100.0)

+ = Positive, - = negative, 0 = no smear

Of the 17 patients giving positive cultures only on MNYC medium A 13 were smear positive, two were smear negative, and in two instances no smears were available. There were no patients giving positive cultures only on TM medium. Smears were positive but cultures negative in 17.1% (13/76) of patients when TM medium was used compared with only 3.9% (3/76) using MNYC medium A. Microscopical examination detected 73.7% (56/76) of all patients with gonorrhoea. Excluding the 10 patients from whom no smears were available, microscopical examination detected 84.8% (56/66) of cases. A combination of microscopy and culture on TM medium detected 90.8% (69/76) whereas culture on TM medium alone detected only 77.6% (56/76). Culture on MNYC medium alone detected 96.1% of men with gonorrhoea.

BOTH MALE AND FEMALE PATIENTS

Rapidity of growth

Colonies of Gram-negative diplococci were present after incubation for 24 hours in 51.8% (29/56) of the cultures positive in women and 42.9% (24/56) of the cultures positive in men when TM medium was used compared with 79.1% (68/86) and 78.1% (57/73) of the cultures respectively when MNYC medium A was used.

MIC determinations

These were carried out on isolates from nine of the 40 patients positive only on MNYC medium A and 14 of the 85 patients positive on both TM and MNYC medium A. The MICs of the nine strains isolated only on MNYC medium A were: vancomycin, 8 µg/ml (three strains), 16 µg/ml (five strains), and ≥32 µg/ml (one strain); lincomycin, 8 µg/ml (two strains), 16 µg/ml (six strains), and ≥32 µg/ml (one strain). The MICs of the 14 strains positive on

both media were: vancomycin, 8 µg/ml (two strains), 16 µg/ml (seven strains), and ≥32 µg/ml (five strains); lincomycin, 8 µg/ml (four strains), 16 µg/ml (seven strains), and ≥32 µg/ml (three strains).

Overgrowth by Proteus spp.

Using TM medium 2.0% of all sites examined in women and 0.5% of sites in men were overgrown with *Proteus* spp. compared with 0.5% and 0.17% of sites respectively when MNYC medium A was used.

Growth of unwanted micro-organisms

This was more pronounced in rectal cultures plated on MNYC medium A than on TM medium. In order to lessen contamination the lincomycin concentration was increased to 1.0 µg/ml for the second part of the study. Because of the expense of horse blood, human blood was also tested in parallel.

STUDIES WITH MNYC MEDIUM B AND MNYC MEDIUM C

MNYC medium B contained 10% horse blood and MNYC medium C 10% human blood: both contained lincomycin (1.0 µg/ml).

Women

Of the 287 female patients examined, 56 gave positive cultures on both media. One additional patient was detected by culture on MNYC medium B.

Men

Of the 417 male patients examined 67 gave positive cultures on both media. One additional patient was detected by culture on MNYC medium B.

Growth of unwanted micro-organisms seemed to be less pronounced with lincomycin (1.0 µg/ml) in the medium. However, this was not a direct comparison with the same medium containing lincomycin (0.5 µg/ml).

Discussion

MNYC medium substantially improved the efficiency and rapidity of the cultural diagnosis of gonorrhoea. In men 96.1% of cases of gonorrhoea were detected by MNYC culture compared with only 77.8% by TM; the corresponding figures for women with gonorrhoea were 100% and 69.0% respectively.

Conventional TM medium has been shown to give poor results in other trials. Willcox and John (1976) found that 91.2% of 102 male patients with positive smears gave positive cultures using the Ames detection kit which contains modified TM

medium, compared with 60.8% positive cultures using conventional TM medium: Ames detection kit or 'Microcult-GC' contains lincomycin, colistin, amphotericin B, and trimethoprim.

The better results with MNYC medium could be because of its superior nutritional value, the replacement of vancomycin with lincomycin, or a combination of these factors. The more rapid growth with MNYC medium demonstrates the improved nutritional state of MNYC medium: after 24 hours of incubation gonococcal colonies were present in 78.6% (125/159) of specimens positive on MNYC medium compared with only 47.3% (53/112) of specimens positive on TM medium. When the inoculum from the patient is low the improved nutrition provided by MNYC medium may be critical in allowing gonococcal growth.

Since none of the strains isolated only on MNYC medium was sensitive to vancomycin at the concentration present in TM medium (4.0 µg/ml) there is no direct support for vancomycin sensitivity as a cause of culture failure with TM medium. Nevertheless, the safety margin with lincomycin is much greater (MIC usually 16 times the concentration present in the medium) than with vancomycin (MIC usually two to four times greater than the corresponding medium concentration). This safety margin combined with the better nutritional value of MNYC medium may be particularly important in the case of small inocula.

Ødegaard *et al.* (1975) found that 'chocolate' (heated blood) agar medium containing lincomycin (0.5 µg/ml) in place of vancomycin (3.0 µg/ml) increased the number of samples positive for gonococci by 7%, and the number of patients with gonococcal infection by 4%: MICs were not reported in this study. These workers also found that the growth of unwanted micro-organisms was more pronounced on the medium containing lincomycin (0.5 µg/ml) in place of vancomycin (3.0 µg/ml).

Trimethoprim lactate did not completely prevent overgrowth by *Proteus* spp. but it reduced the problem considerably. It did not appear to have any inhibitory effect on gonococci since no isolates were obtained only on TM medium lacking trimethoprim.

MNYC medium was not compared directly with the NYC medium described by Faur *et al.* (1973a, b) and it is therefore impossible to assess the performance of the modified medium with the original. The comparison with our conventional TM medium and the very low proportion of smear-positive culture negative patients using MNYC medium suggests that the modified medium performs extremely well. The modified medium is much

simpler to prepare than the original since it uses a commercially available base (Difco gonococcal base). Faur *et al.* (1973a) reported that better results were obtained when the proteose peptone-cron starch agar buffered base was prepared from basic ingredients although quantitative data were not presented. The other major simplification in the MNYC medium is the use of completely lysed whole blood in place of fresh plasma and haemoglobin solution.

Faur *et al.* (1976) stated that if horse blood was not available cow blood could be substituted in MNYC medium, but that sheep blood was unsuitable: 92 of 522 cultures for *N. gonorrhoeae* were positive on both NYC media supplemented with either horse blood or cow blood, but only 85 positive cultures were obtained using sheep blood. Owing to the expense of horse blood and the small difference in the yield of positive cultures when human blood is used, this is considered to be an acceptable alternative to horse blood for the cultural diagnosis of gonorrhoea.

As a result of these findings, MNYC medium containing 10% human blood and lincomycin (1.0 µg/ml) was introduced in February 1977 for the routine isolation of gonococci.

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