Improved Medium Selective for Cultivation of *N. Gonorrhoeae* and *N. Meningitidis*

JAMES D. THAYER, Ph.D., and JOHN E. MARTIN, Jr., B.A.

DEVELOPMENT of a selective medium for the cultivation of gonococci and meningococci was reported by Thayer and Martin in 1964 (*1*). The Thayer-Martin (T-M) medium has been widely accepted for primary isolation of the gonococcus and meningococcus from sites where these organisms are far outnumbered by the natural bacterial flora. Overgrowth by gram-positive and gram-negative bacteria is prevented by addition of 10 micrograms of ristocetin and 25 units of polymyxin B per ml. of conventional diagnostic medium. In this concentration, the antibiotics also prevent growth of the saprophytic *Neisseria*.

In 1964 ristocetin (Abbott Laboratories, Chicago) was removed from the market. The step caused considerable concern, and it was necessary to find a suitable substitute.

Presumptive positive cultural diagnosis had become possible for gonorrhea and the meningococcal carrier state with the use of the T-M selective medium. Such presumptive evidence had been confirmed in a vast number of instances by fermentation and fluorescent antibody procedures.

As a means of screening for asymptomatic carriers of meningococci, the immunofluorescent staining of material from the nasopharynx was found by Mitchell and co-workers (*2*) to be less satisfactory than cultivation with this selective medium.

In confirming the findings of Thayer and coworkers (*3*) Van Peenen and associates (*4*) showed that with the T-M medium two to four times as many nasopharyngeal carriers of meningococci were identified as with the Mueller-Hinton medium. The growth of other organisms often obscured the meningococci on the Mueller-Hinton medium. Van Peenen also agreed with Mitchell that T-M plates with fewer than 10 colonies usually did not contain meningococci and that those with more than 10 colonies almost always contained meningococci. The T-M medium was found to be excellent for large carrier surveys, superior in both sensitivity and specificity to conventional media.

Wende and associates (*5*) found 18 percent more isolations of *Neisseria gonorrhoeae* from patients with clinical infection assumed to be gonorrhea with the T-M medium than with modified Lankford medium. When ristocetin and polymyxin B were added to MacLeod's chocolate agar, Wilkinson (*6*) obtained a 10 percent increase in the number of patients in whom a full bacteriological diagnosis of gonorrhea could be made. He also noted that the markedly suppressed growth of contaminating organisms

Dr. Thayer is chief and Mr. Martin is a bacteriologist, Antibiotics Surveillance and Methodology Laboratory, Venereal Disease Branch, Communicable Disease Center, Public Health Service, Atlanta, Ga.

facilitated the isolation of gonococci in pure culture. Further, the selective medium was found to be of great value when dealing with rectal cultures.

In further studies with the selective medium, Martin and co-workers (*7*) concluded that diagnosis of vaginal and rectal specimens was feasible and that the percentage of positives from these sites and the cervical site was improved. The number of specimens positive on T-M medium after 40 hours of incubation was greater than that obtained by the delayed fluorescent antibody procedure. This was thought to be due to suppression of contaminating bacteria and to the longer growth period.

The high degree of specificity and selective sensitivity of the T-M selective medium returned the culture method to favor for diagnosing gonorrhea, and it has been enthusiastically accepted for rapid, accurate epidemiologic investigation of the asymptomatic meningococcal carrier state in large populations.

The withdrawal of ristocetin, used to suppress gram-positive contaminants, from the market prompted a search for a substitute medium. The substitute is an improved medium, with greater selective and inhibitory properties than those of the medium previously advocated. The new antibiotic supplement uses vancomycin to inhibit gram-positive contaminants, colistimethate sodium for the gram-negative bacterial flora, and nystatin to inhibit yeast.

Methods

Media. A conventional chocolate agar (Bacto GC medium base with added hemoglobin and yeast supplement B) was used as a base medium to which antibiotics were added in the concentrations given in table 1. The antibiotics, supplied by the manufacturers (*A–C*), had the following potencies:

Antibiotic	*Lot No.*	*Potency*
Vancomycin sulfate (*A*)	PA85497	962 units/mg.
Colistimethate sodium (*B*)	0127014	1,000 μg./mg.
Nystatin (*C*)	4K898	500,000 units/vial.

Cultures. Lyophilized pure stock strains of *Neisseria* and other bacteria were used to determine the antibiotic concentrations to be used in the medium. Bacterial inoculum was standardized as before by Thayer and Martin in 1964 (*1*).

Results

The tolerance of gonococcal strains for vancomycin and colistimethate is given in table 1. The 57 stock strains were uninhibited by 7.5 units/ml. or less of vancomycin, and colistimethate did not inhibit growth until a concentration of 20 μg./ml. was reached. None of 76 gonococcal strains were inhibited by 12.5 units/ml. of nystatin, the highest concentration tested.

To determine whether vancomycin and colistimethate acting together in the medium might antagonize their effect for contaminants or by synergism inhibit gonococci, a checkerboard titration was carried out by using several concentrations of both drugs. Sets of 12 concentration combinations were inoculated with 40 different specimens obtained from 24 men and 16 women with gonorrhea. The concentration combination that seemed most suitable for suppressing contamination and allowing gonococcal strains to grow uninhibited after 20 hours of incubation were: 3 units vancomycin, 7.5 μg. colistimethate, and 12.5 units nystatin per ml. of medium.

Table 1. Sensitivity of *Neisseria gonorrhoeae* to vancomycin and colistimethate, by number of strains that grew comparable with controls

Concentration of antibiotic	Number of strains
Vancomycin (unit/ml.)	(1)
20	39
15	46
10	51
7.5	57
5	57
3	57
0	57
Colistimethate (μg./ml.)	(2)
25	77
20	77
15	78
12.5	78
10	78
7.5	78
0	78

[1] Total 57 strains.
[2] Total 78 strains.

Table 2. Selective growth of *Neisseria* and other micro-organisms on three media

Organism	Number of strains	Media		
		GC chocolate agar	Polymyxin-ristocetin [1]	Vancomycin-colistimethate-nystatin [2]
Staphylococcus aureus	6	6	0	0
Staphylococcus epidermidis	7	7	4	0
Sarcina lutea	2	2	0	0
Escherichia coli	1	1	0	0
Pseudomonas aeruginosa	2	2	0	0
Pseudomonas fluorescens	1	1	0	0
Bacillus species	3	3	0	0
Corynebacterium species	3	3	0	0
Listeria monocytogenes	2	2	0	0
Lactobacillus species	3	3	1	1
Mima polymorpha	3	3	1	1
Mima polymorpha variant *oxidans*	2	2	0	0
Herellea vaginicola	3	3	0	0
Neisseria sicca	5	5	0	0
Neisseria flava	3	3	0	0
Neisseria perflava	4	4	0	0
Neisseria subflava	2	2	0	0
Neisseria flavescens	4	4	0	0
Neisseria catarrhalis	6	6	3	1
Neisseria meningitidis A	2	2	2	2
Neisseria meningitidis B	1	1	1	1
Neisseria meningitidis C	1	1	1	1
Neisseria meningitidis D	1	1	1	0

[1] PR=25 units polymyxin B, 10 μg. ristocetin per ml. of medium.
[2] VCN=3 units vancomycin, 7.5 μg. colistimethate, 12.5 units nystatin per ml. of medium.

Table 2 gives the results obtained when polymyxin B-ristocetin (PR), vancomycin-colistimethate-nystatin (VCN), and chocolate agar were inoculated with pathogenic and saprophytic *Neisseria* and with gram-positive and gram-negative bacteria. The VCN selective medium inhibited more *Staphylococcus epidermidis* and *Neisseria catarrhalis* than the PR medium. Only the group D meningococcus failed to grow on VCN medium. One of three strains of *Mima polymorpha* grew on both selective media, but the *oxidans* variant failed to grow. Meningococci were uninhibited, but *Mima polymorpha* variant *oxidans* and other natural inhabitants of the nasopharynx, vagina, and rectum failed to grow or were greatly inhibited.

Little difference could be noted in the efficacy of PR and VCN selective media when compared by inoculating plates of each with 0.1 ml. of inoculum prepared from 112 vaginal specimens. The PR medium isolated 45 and the VCN medium 49 of a total of 51 positive gonococcal cultures. There was agreement in 43 cultures; 6 were positive on VCN and negative on PR, and 2 were positive on PR and negative on VCN media. Spreading proteus-like growth was uninhibited by either medium in six specimens. Only an occasional yeast contaminant was noted among the vaginal specimens on the VCN medium.

Discussion

The antibiotics vancomycin sulfate and colistimethate sodium have been successfully substituted for ristocetin and polymyxin B as inhibitors of bacterial contaminants and saprophytic *Neisseria* in the selective medium for gonococci and meningococci previously developed by Thayer and Martin. Colistimethate proved to be more inhibitory for saprophytic *Neisseria* than polymyxin B. The addition of nystatin resulted in inhibiting some yeast strains present in vaginal specimens.

There was little difference in the amount of contamination or the number of positive isolations of gonococci in either medium. Gonococcal growth became evident earlier on the VCN medium, and micrococci were more strongly inhibited. The effectiveness of VCN for suppressing growth of *N. catarrhalis* and other saprophytic *Neisseria*, seldom encountered in vaginal specimens, must await field trials in epidemiologic surveys for meningococcal carriers.

Summary

The Thayer-Martin (T–M) selective medium for gonococci and meningococci has been widely accepted for the primary isolation of these organisms from conspicuously contaminated sites. The high degree of specificity and selective sensitivity of the medium also made it possible to accept with assurance presumptive culture testing for gonorrhea and the meningococcal carrier state.

Because ristocetin, used in the medium to sup-

press growth of the gram-positive flora, was removed from the market in 1964, it was necessary to find a suitable substitute. The new antibiotic supplement offered uses vancomycin to inhibit gram-positive contaminants, sodium colistimethate for the gram-negative flora, and nystatin to inhibit yeast, which is sometimes a nuisance in vaginal and rectal cultures.

Comparison of the new medium with its predecessor showed equivalent growth of gonococci in specimens obtained from men and women with gonorrhea and greater inhibition of coagulase positive and negative staphylococci and of saprophytic *Neisseria*.

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(*6*) Wilkinson, A. E.: A note on the use of Thayer and Martin's selective medium for *Neisseria gonorrhoeae*. Brit J Vener Dis 41: 60–61 (1965).

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SUPPLY REFERENCES

(*A*) Eli Lilly & Co., Indianapolis, Ind.

(*B*) Warner-Chilcott Laboratories, Morris Plains, N.J.

(*C*) E. R. Squibb & Sons, New Brunswick, N.J.

Investigational Vaccines Program

The Communicable Disease Center has established a program to provide vaccines that are needed for human immunization but are not available from commercial sources. The objective is to provide qualified investigators with a source of vaccines which are of established effectiveness and safety, but have applications too limited to sustain commercial production and distribution. These steps are being taken on the recommendation of the Public Health Service Advisory Committee on Immunization Practice.

The first product that will be available in this program is the pentavalent (ABCDE) botulinum toxoid, aluminum phosphate adsorbed, developed by Dr. George G. Wright and associates at the U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md.

The toxoid will be distributed as an investigational new drug. Inquiries and requests for this item should be addressed to: Investigational Vaccines Program, Laboratory Branch, Communicable Disease Center, Atlanta, Ga. 30333.

Suggestions will be considered regarding additional prophylactic agents that meet the program objective and which might be considered for provision by the Communicable Disease Center in the future.