

Use of New York City Medium for Improved Recovery of *Neisseria gonorrhoeae* from Clinical Specimens

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New York City (NYC) and Martin-Lewis (ML) media were evaluated comparatively for their ability to support the growth of *Neisseria gonorrhoeae* from clinical specimens. A total of 1,010 urethral, cervical, pharyngeal, and rectal specimens were collected from walk-in patients attending a clinic for sexually transmitted diseases. A total of 187 and 165 isolates of gonococci were cultivated on NYC and ML media, respectively, with 161 of these isolates being recovered on both media. Overall, the use of NYC medium resulted in a 13.3% increased recovery rate of gonococci. When gonococci were recovered on both media from primary isolation, the NYC medium supported a more luxuriant growth and a greater number of colonies, which usually resulted in the detection of positive cultures 1 day sooner than on ML medium. Both media were comparable in their ability to suppress the growth of saprophytic microorganisms. The results of this study demonstrated that the use of NYC medium markedly enhanced the recovery of *N. gonorrhoeae* from clinical specimens as compared to ML medium.

Although gonorrhea is still considered a disease of epidemic proportions, the annual total of reported cases of this infection in the United States has not increased significantly since 1975 (2). An important contributing factor which has helped to control the incidence of this disease has been the widespread use of specialized media for the enhanced recovery of *Neisseria gonorrhoeae* from polymicrobial specimens.

The development of Thayer-Martin medium (26) in 1964 represented the first major advance for the cultural diagnosis of gonorrhea. Since that time, the original Thayer-Martin medium has undergone a number of modifications and improvements (3, 16, 17, 20-23, 29), with Martin-Lewis (ML) medium (15) representing the most recent modification in the original Thayer-Martin formulation. Currently, ML medium is used in many laboratories for the primary isolation of *N. gonorrhoeae*. This has resulted in the improved recovery of gonococci from clinical specimens.

In 1973, Faur and her colleagues described a new gonococcal selective isolation medium (6, 7, 10), called New York City (NYC) medium. Evaluation of this medium has shown it to be comparable (11, 24, 25, 30) to improved modifications of Thayer-Martin agar for the recovery of gonococci from clinical material. In addition, NYC medium has been reported to have the added

advantage of supporting the growth of urogenital mycoplasmas (8, 11).

In the mid-1970s, several investigators (1, 5, 18) showed that the concentration of vancomycin used in either ML or NYC medium may be inhibitory to the growth of as many as 10% of gonococcal strains. As a result of these reports, Faur and her co-workers compared the effects of reduced concentrations of vancomycin in NYC medium on the recovery of gonococci from clinical specimens. The results of their study (9) showed that the recovery rate of *N. gonorrhoeae* could be appreciably improved without a concomitant increase in contamination rates by reducing the concentration of vancomycin in NYC medium.

Since the use of this improved NYC (INYC) medium resulted in increased recovery rates of *N. gonorrhoeae* as compared to the original NYC formulation, it seemed possible that INYC medium might provide a significant advantage over the widely used ML agar for the isolation of gonococci. The purpose of this prospective study is to evaluate INYC and ML media for their comparative value in the recovery of *N. gonorrhoeae* from clinical specimens.

MATERIALS AND METHODS

Media. NYC medium contains a proteose-peptone-cornstarch agar buffered base enriched with horse

plasma, yeast dialysate, dextrose, and a solution of 3% lysed horse erythrocytes. The antimicrobial selective agents included vancomycin (3 µg/ml), colistin (7.5 µg/ml), amphotericin B (1 µg/ml), and trimethoprim lactate (3 µg/ml). INYC medium was prepared in-house as originally described by Faur and her associates (6, 7, 10), except that the vancomycin concentration was reduced to 2 µg/ml (9).

ML medium consists of an enriched chocolate agar containing the antimicrobial inhibitors vancomycin (4 µg/ml), colistin (7.5 µg/ml), anisomycin (20 µg/ml), and trimethoprim lactate (5 µg/ml). This medium was made according to the formulation described by Martin and Lewis (15) and was obtained commercially (Tidewater Biologicals, Inc., Chesapeake, Va.).

The INYC and ML media were prepared in Jembec plates (Lab-Tek Products, Naperville, Ill.) (14), and each batch or lot number of medium was performance-tested before use for its ability to support the growth of *N. gonorrhoeae* as well as to inhibit the growth of *Escherichia coli* and enterococci. All media were stored at 4°C and warmed to room temperature before use.

Speciman collection and processing. A total of 1,010 clinical specimens were collected from walk-in patients attending the Sexually Transmitted Disease Clinic at the Onondaga County Department of Health, Syracuse, N.Y. Clinical samples were collected by trained nurses from urethra, cervix, pharynx, or rectum, or a combination of these. Urethral specimens were collected with calcium alginate-tipped applicators (Becton Dickinson & Co., Rutherford, N.J.), whereas specimens from the other anatomic sites were collected with cotton-tipped swabs. In addition, a separate urethral swab specimen was collected from males with a purulent discharge to prepare a smear for Gram stain examination.

Swab specimens that were collected for culture were placed in tubes containing 0.7 ml of a sterile 0.4% gelatin solution in distilled water. The swab was twirled in the solution to elute the clinical material, rimmed against the side of the tube to express the excess fluid, and then discarded. Each of the two test media was inoculated with a separate applicator that was dipped into the sample solution. The swab was rolled slowly over the surface of the test medium in a large "Z" pattern to maximize transfer of the microorganisms to the agar surface. A carbon dioxide-generating tablet was placed in the well of each Jembec chamber, and each plate was sealed in an individual plastic "zip-lock" environmental pouch and immediately placed in a 35°C incubator.

All Jembec plates were incubated for 72 h and examined daily for the appearance of microbial growth. Bacterial isolates were identified as *N. gonorrhoeae* on the basis of colonial morphology, Gram stain, oxidase reaction, and pattern of carbohydrate utilization in cystine-tryptophan agar media.

Vancomycin susceptibility testing. Isolates of *N. gonorrhoeae* which were recovered only on INYC medium were saved for additional studies. A heavy gonococcal suspension was prepared from an actively growing isolate on a chocolate agar plate and transferred to a tube containing tryptic soy broth with 30%

glycerol. The bacterial suspensions were quick-frozen in a dry ice-ethanol bath and subsequently stored at -50°C until ready for use.

Vancomycin susceptibility testing was performed on the gonococcal isolates by a standardized agar dilution procedure (28). Each gonococcal isolate was tested against onefold dilutions of vancomycin ranging from 1 to 10 µg/ml. The minimum inhibitory concentration (MIC) for each isolate was determined as the lowest concentration of vancomycin which completely suppressed visible growth after 48 h of incubation. A *Staphylococcus aureus* strain and an enterococcus strain with vancomycin MICs of 2.0 µg/ml and less than 1.0 µg/ml, respectively, were used as the quality control reference strains.

Adaptability study. The strains of *N. gonorrhoeae* which were recovered only on INYC medium were subsequently tested for their ability to grow on ML agar. Using actively growing strains from a chocolate agar plate, a McFarland 0.5 standard density suspension was prepared in 5 ml of tryptic soy broth for each gonococcal isolate. A calibrated loop was used to transfer a 0.001-ml inoculum onto each surface of INYC and ML media. The plates were incubated at 35°C with 5% carbon dioxide and examined comparatively after 24 and 48 h of incubation for relative numbers and size of gonococcal colonies.

Statistical analysis. The comparative results obtained from this study were analyzed by the chi-square test of dissimilar pairs (4). A χ^2 value of 3.8 or greater is regarded as significant.

RESULTS

Table 1 shows the comparative recovery rates of *N. gonorrhoeae* on the INYC and ML media from the 1,010 clinical samples processed in this study. A total of 191 gonococcal isolates were recovered from these specimens; 161 strains were isolated on both media. Of the 30 discrepant isolates, 26 strains were recovered only on the INYC medium, whereas the remaining four isolates grew only on the ML agar. Interestingly, cervical cultures accounted for only 20% of the total number of specimens processed in this study, but 42% of the gonococcal isolates that were cultivated on INYC medium only were recovered from this anatomic site.

TABLE 1. Comparison of gonococcal recovery rates on INYC and ML media

Source	No. of specimens cultured	No. of gonococci recovered on:		
		Both media	INYC medium only	ML medium only
Urethra	518	110 (21.2)	12 (2.3)	0 (0)
Cervix	197	40 (20.3)	11 (5.6)	3 (1.5)
Pharynx	230	5 (2.2)	2 (0.9)	0 (0)
Rectum	65	6 (9.2)	1 (1.5)	1 (1.5)
Total	1,010	161 (15.9)	26 (2.6)	4 (0.4)

^a Numbers within parentheses denote percent positive cultures.

Overall, the use of INYC medium resulted in 187 gonococcal isolations, or a 13.3% increased recovery rate as compared to ML agar. Analysis of these data showed that the results were statistically significant ($\chi^2 = 17.63$).

Figures 1 and 2 show the comparative colonial growth of *N. gonorrhoeae* from a representative clinical sample after 24 and 48 h of incubation. The luxuriant growth of gonococci on the INYC medium usually resulted in reduced incubation times to detect positive cultures. Of the 187 gonococcal strains which grew only on INYC medium, 86% of the isolates were detected within the first 24 h of incubation, and the remaining isolates were recovered after an additional day of incubation. Detection of growth for most isolates recovered on ML agar usually required 2, and in some cases 3, days of incubation. These colonies were invariably smaller in size and usually fewer in number as compared to the same specimen inoculated onto INYC medium.

Although the INYC medium supported a more luxuriant growth of gonococci than ML agar, both media were comparable in their selective ability to inhibit the growth of commensal microorganisms. Even though the mixture of antimicrobial agents in each of the media was slightly different and the INYC medium contained a lower concentration of vancomycin, the contamination rate observed on each of the media was below 3%.

The 26 strains of *N. gonorrhoeae* which grew only on the INYC medium were recovered from

25 patients (gonococci were recovered from both the urethra and pharynx of one patient). The clinical presentation of these patients and other pertinent data are presented in Table 2. The male-female sex distribution was comparable in this group, and the majority of patients (64%) were symptomatic from their infection. The specimen sources included 12 urethral, 11 cervical, 2 pharyngeal, and 1 rectal. Gram stain smears were prepared on 8 of the 12 urethral specimens, and gram-negative intracellular diplococci were observed in 7 of these 8 smears.

The clinical presentations of the four patients from whom gonococci were recovered only on ML agar are shown in Table 2. All four patients were recent contacts of partners with documented infection, and the majority of patients were symptomatic females.

Nineteen of the 26 gonococcal isolates which were recovered only on the INYC medium were tested for their susceptibility to vancomycin by a standardized agar dilution method (28). The results showed that the higher concentration of vancomycin (4 $\mu\text{g}/\text{ml}$) in the ML agar was not responsible for the failure of these organisms to grow on this medium. All of the gonococci tested had vancomycin MICs considerably greater than the 4 $\mu\text{g}/\text{ml}$ present in ML medium. Seventeen of the gonococcal isolates had MICs greater than 10 $\mu\text{g}/\text{ml}$, and the remaining two strains had identical MICs of 7 $\mu\text{g}/\text{ml}$.

These same 19 isolates of gonococci that were recovered only on INYC medium were tested for their ability to grow on ML agar. A calibrated

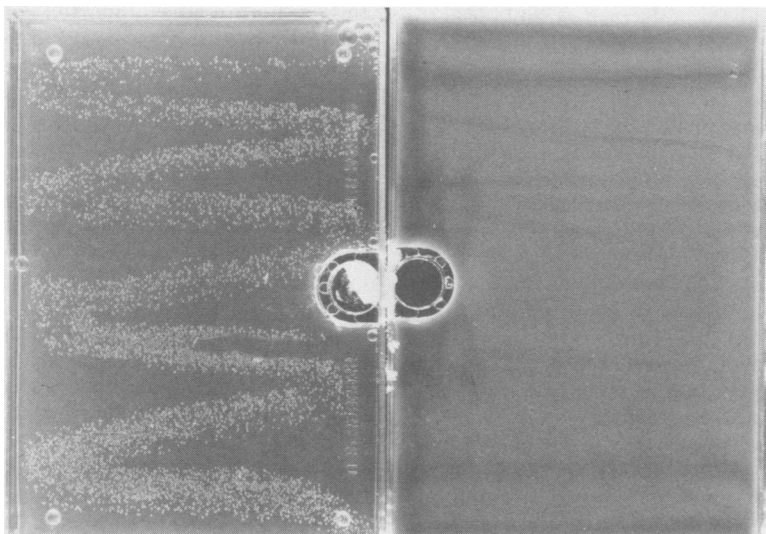


FIG. 1. Comparative growth of *N. gonorrhoeae* isolated from a typical clinical specimen on INYC (left) and ML (right) media after 24 h of incubation.

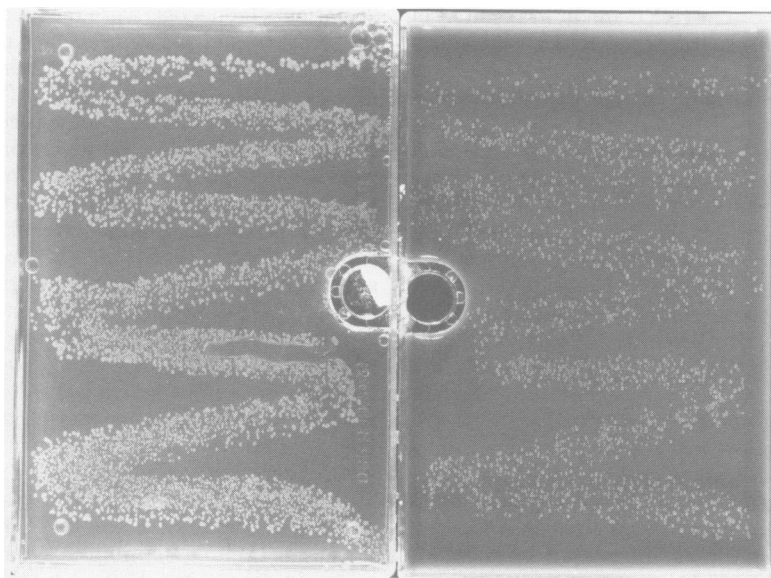


FIG. 2. Comparative growth of *N. gonorrhoeae* isolated from a typical specimen on INYC (left) and ML (right) media after 48 h of incubation.

loop was used to transfer a 0.001-ml inoculum from a standardized suspension onto the surfaces of INYC and ML media. Growth of all isolates tested was detected on each of the media after 24 h of incubation. For each isolate studied, the size and relative number of colonies produced were identical on each medium.

DISCUSSION

The experimental protocol of this study was designed so that the only test variable was the INYC and ML media. Each clinical specimen was collected on a swab and eluted into a diluent, and separate swabs were dipped into the resultant microbial suspension to transfer a standardized inoculum to each of the test media. The conditions of specimen transport and the criteria for cultural workup were identical.

The results of this study clearly showed that the use of INYC medium significantly enhanced the recovery of *N. gonorrhoeae* from clinical specimens as compared to ML agar. Since several investigators (1, 5, 18) have shown that the 4 μ g of vancomycin per ml used in ML medium may be inhibitory to the growth of 10% of all gonococcal strains, one might reasonably predict that the lower concentration of vancomycin used in INYC medium might be responsible for the 13.3% improved isolation rate of *N. gonorrhoeae* observed in this study. However, the results of the vancomycin agar dilution susceptibility studies showed that the enhanced recovery of gonococci on the INYC medium was not attributable

to the lower concentration of this drug in the medium. All of the isolates tested had MICs considerably greater than the vancomycin concentration present in ML medium.

This observation was supported by the adaptability study, which comparatively evaluated the ability of standardized suspensions of gonococci to grow on INYC and ML media. All of the 19 isolates tested which were originally recovered only on INYC medium grew on the ML medium. In fact, both media supported comparable gonococcal growth, with colonies of equivalent size and numbers within 24 h of incubation. This finding was unexpected because the isolation of gonococci on both media directly from a clinical specimen was invariably more luxuriant, with a greater number of colonies, on INYC than on ML medium. It appears that once the gonococcus has been isolated from primary culture it becomes adapted to grow on other types of laboratory media. This microbial phenomenon of adaptability may have misled some investigators to conclude that INYC and ML media were comparable in their ability to support the growth of *N. gonorrhoeae* if the in-house media evaluations were performed with stock laboratory isolates of gonococci.

The improved growth of gonococci on INYC medium from clinical specimens may be attributable to the supplemental enrichments used in this medium. INYC medium consists basically of a proteose-peptone agar base enriched with horse plasma, yeast dialysate, and lysed horse

TABLE 2. Summary of patients who had gonococci recovered on INYC or ML medium only

Gonococci recovered on:	Patient no./sex	Clinical referral	Last contact (days)	Days of symptoms	Specimen source	Gram stain smear ^a
INYC medium	1/M	Self	10	3-4	Urethra	+
	2/M	Self	7	6	Urethra	ND
	3/M	Self	7	Asym ^b	Urethra	ND
	4/M	Self	3	7	Urethra	+
	5/M	Contact	4	14	Urethra	ND
	6/M	Self	3	3	Urethra	+
	7/M	Self	5	1	Urethra	+
	8/M	Self	1	10	Urethra/pharynx	+
	9/M	Contact	6	Asym	Rectum	ND
	10/M	Contact	14	Asym	Urethra	-
	11/M	Self	7	3	Urethra	+
	12/M	Self	6	3	Urethra	+
	13/M	Contact	3	Asym	Urethra	ND
	14/F	Contact	2	Asym	Cervix	ND
	15/F	Contact	4	2	Cervix	ND
	16/F	Contact	2	2	Cervix	ND
	17/F	Contact	7	7	Cervix	ND
	18/F	Self	1	1	Cervix	ND
	19/F	Contact	4	Asym	Cervix	ND
	20/F	Contact	14	7	Cervix	ND
	21/F	Contact	7	120	Cervix	ND
	22/F	Contact	5	Asym	Pharynx	ND
	23/F	Contact	2	7	Cervix	ND
	24/F	Contact	14	Asym	Cervix	ND
	25/F	Contact	1	Asym	Cervix	ND
ML medium	1/F	Contact	1	21	Cervix	ND
	2/F	Contact	1	2	Cervix	ND
	3/F	Contact	2	Asym	Cervix	ND
	4/M	Contact	10	180	Rectum	ND

^a +, Positive; ND, not done; -, negative.

^b Asym, Asymptomatic.

erythrocytes. The enhanced growth of gonococci on INYC medium from clinical specimens may result from horse plasma or yeast dialysate or both; both are absent in the formulation of ML medium. The addition of animal sera to laboratory media has been recognized for many years as an important growth-promoting factor for fastidious microorganisms (19). Similarly, the presence of yeast extract or yeast dialysate in media has been shown to stimulate the growth of *N. gonorrhoeae* from clinical specimens even when such media are incubated in ambient air (12, 13). Reportedly, yeast extract contains oxaloacetic acid, which is metabolized by the gonococcus to produce sufficient amounts of carbon dioxide, thus supporting the continued growth of the capnophilic gonococcus on the medium (13).

In the recovery of *N. gonorrhoeae* from clinical specimens, it is well recognized that there can be significant loss of viable microorganisms during their physiological adjustment to the growth conditions on an artificial medium. The presence of horse plasma or yeast dialysate or

both in the INYC medium may minimize the organism's physiological shock to the artificial medium, resulting in a smaller loss of viable microorganisms. Conceivably, this hypothesis could explain the improved growth and increased recovery of *N. gonorrhoeae* from clinical specimens on the INYC medium as compared to ML agar. In addition, this same hypothesis may account for the disproportionate number of gonococcal isolates that were recovered from cervical cultures on INYC medium only.

In summary, this study has shown that the use of INYC medium resulted in a 13% increased recovery of *N. gonorrhoeae* from clinical specimens as compared to ML medium. The widespread use of this medium should result in an improved detection system for the continued control of gonorrhea, with the goal of reduction in the incidence of this disease.

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LITERATURE CITED

1. **Brorson, J. E., J. Holmberg, B. Nygren, and S. Seeberg.** 1973. Vancomycin-sensitive strains of *Neisseria gonorrhoeae*. A problem for the diagnostic laboratory. *Br. J. Vener. Dis.* **49**:452-453.
2. **Center for Disease Control.** 1979. Gonorrhoea—United States. *Morbidity and Mortality Weekly Report* **28**:533-534.
3. **Chen, N. C., S. S. Hipp, W. D. Lawton, and H. A. Gaafar.** 1974. Gonogrow, an improved, selective medium for the isolation of *Neisseria gonorrhoeae*. *Health Lab. Sci.* **11**:173-177.
4. **Colton, T.** 1974. *Colton statistics in medicine*, p. 177-179. Little, Brown and Co., Boston.
5. **Cross, R. C., M. B. Hager, R. Neibaur, B. Pasternak, and F. J. Brady.** 1971. VCN-inhibited strains of *Neisseria gonorrhoeae*. HSMHA Health Rep. **86**:990-992.
6. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1973. A new medium for the isolation and pathogenic *Neisseria* (NYC medium). II. Effect of amphotericin B and trimethoprim lactate on selectivity. *Health Lab. Sci.* **10**:55-60.
7. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1973. A new medium for the isolation of pathogenic *Neisseria* (NYC medium). III. Performance as a culture and transport medium without addition of ambient carbon dioxide. *Health Lab. Sci.* **10**:61-74.
8. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Morphologic observations of mycoplasmas and *Neisseria gonorrhoeae* in associated growth patterns. *Am. J. Clin. Pathol.* **63**:106-116.
9. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1978. The selectivity of vancomycin and lincomycin in NYC medium for the recovery of *N. gonorrhoeae* from clinical specimens. *Health Lab. Sci.* **15**:22-27.
10. **Faur, Y. C., M. H. Weisburd, M. E. Wilson, and P. S. May.** 1973. A new medium for the isolation of pathogenic *Neisseria* (NYC medium). I. Formulation and comparisons with standard media. *Health Lab. Sci.* **10**:44-54.
11. **Faur, Y. C., M. H. Weisburd, M. E. Wilson, and P. S. May.** 1974. NYC medium for simultaneous isolation of *Neisseria gonorrhoeae*, large-colony mycoplasmas, and T-mycoplasmas. *Appl. Microbiol.* **27**:1041-1045.
12. **Faur, Y. C., M. H. Weisburd, M. E. Wilson, and P. S. May.** 1977. Field evaluation of New York City medium in the biological environment-CO₂ chamber in recovery of *Neisseria gonorrhoeae* and urogenital mycoplasmas. *J. Clin. Microbiol.* **5**:137-141.
13. **Griffin, P. J., and S. V. Rieder.** 1957. A study on the growth requirements of *Neisseria gonorrhoeae* and its clinical application. *Yale J. Biol. Med.* **29**:613-21.
14. **Martin, J. E., J. H. Armstrong, and P. B. Smith.** 1974. New system for cultivation of *Neisseria gonorrhoeae*. *Appl. Microbiol.* **27**:802-805.
15. **Martin, J. E., Jr., and J. S. Lewis.** 1977. Anisomycin: improved antimycotic activity in modified Thayer-Martin medium. *Public Health Lab.* **35**:53-62.
16. **Martin, J. E., Jr., and A. Lester.** 1971. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHA Health Rep. **86**:30-33.
17. **Odegaard, K.** 1971. Trimethoprim for the prevention of overgrowth by swarming *Proteus* in the cultivation of gonococci. *Acta Pathol. Microbiol. Scand. Sect. B* **79**:545-548.
18. **Odegaard, K., O. Solberg, J. Lind, G. Myhre, and B. Nyland.** 1975. Lincomycin in selective medium for the isolation of *Neisseria gonorrhoeae*. *Acta Pathol. Microbiol. Scand. Sect. B* **83**:301-304.
19. **Peizer, L. R.** 1939. A method of employing horse plasma and hemoglobin as enrichments in primary gonococcus isolations. *J. Lab. Clin. Med.* **25**:299-303.
20. **Reyn, A.** 1969. Recent developments in the laboratory diagnosis of gonococcal infections. *Bull. W.H.O.* **40**:245-253.
21. **Reyn, A., and M. W. Bentley.** 1972. Comparison of a selective and nonselective medium in the diagnosis of gonorrhoea to ascertain the sensitivity of *Neisseria gonorrhoeae* to vancomycin. *Br. J. Vener. Dis.* **48**:363-368.
22. **Ridell, R. H., and A. C. Buck.** 1970. Trimethoprim as an additional selective agent in media for the isolation of *N. gonorrhoeae*. *J. Clin. Pathol.* **23**:481-483.
23. **Seth, A.** 1970. Use of trimethoprim to prevent overgrowth by *Proteus* in the cultivation of *N. gonorrhoeae*. *Br. J. Vener. Dis.* **46**:201-202.
24. **Symington, D. A.** 1975. An evaluation of New York City transport medium for the detection of *Neisseria gonorrhoeae* in clinical specimens. *Health Lab. Sci.* **12**:69-75.
25. **Symington, D. A.** 1975. Improved transport system for *Neisseria gonorrhoeae* in clinical specimens. *J. Clin. Microbiol.* **2**:498-503.
26. **Thayer, J. T. D., and J. E. Martin, Jr.** 1964. A selective medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Public Health Rep.* **79**:49-57.
27. **Thayer, J. T. D., and J. E. Martin.** 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Health Lab. Rep.* **81**:559-562.
28. **U. S. Department of Health, Education and Welfare.** 1963. *Gonococcus: procedures for isolation and identification*. Publication 499, Public Health Service. U. S. Government Printing Office, Washington, D.C.
29. **Whitbeck, E., N. Shemansky, and M. E. Levison.** 1975. Superiority of amphotericin over nystatin in Thayer-Martin medium. *Antimicrob. Agents Chemother.* **7**:658-660.
30. **Young, H.** 1978. Cultural diagnosis of gonorrhoea with modified New York City (mNYC) medium. *Br. J. Vener. Dis.* **54**:36-40.