

## Isolation of *Neisseria gonorrhoeae* on Selective and Nonselective Media in a Sexually Transmitted Disease Clinic

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To assess the practical significance of reported increases in the prevalence of vancomycin-susceptible strains of *Neisseria gonorrhoeae* on isolation of this organism, antibiotic-free chocolate agar (CA), modified Thayer-Martin medium (MTM), and a vancomycin-free selective medium (VFSM) were compared in a sexually transmitted disease clinic. Among 326 cervical gonococcal infections detected in a comparison of CA with MTM, 92.0% were detected on CA, compared with 98.2% on MTM ( $P < 0.001$ ). Similarly, among 306 cervical infections detected in a comparison of MTM and VFSM, 95.8% of infections were detected with VFSM, compared with 98.4% for MTM ( $P = 0.10$ ). For 1,632 urethral infections in men, all three media were equivalent, with none detecting fewer than 98% of the infections. Compared with a single inoculation, dual inoculation of MTM increased the diagnostic yield by 1.5% for 206 urethral infections and 2.4% for 83 cervical infections. In our clinic population, MTM is superior to CA or VFSM for the diagnosis of genital gonococcal infections, especially in women. The increased yield that accrued from inoculation of both MTM and either of the other media was not sufficiently high to warrant routine use of this practice in our clinic.

The recovery of *Neisseria gonorrhoeae* from the endocervix, anal canal, or pharynx frequently is hampered by overgrowth of other organisms that may mask the presence of gonococci or inhibit their growth by producing specific inhibitors (8, 13, 16, 26), and the incorporation of vancomycin and other antimicrobial agents in media for the primary isolation of pathogenic *Neisseria* sp. (17, 18, 22, 24, 25) was a major advance in the diagnosis of gonorrhea. More recently, however, strains of *N. gonorrhoeae* with increased susceptibility to vancomycin have evolved, and they now account for 0.3 to 30% of gonococcal isolates in various geographic areas (1, 3, 10, 15, 19, 21, 23, 25a, 27). Although such strains may be inhibited in vitro by the concentrations of vancomycin used in most selective media (2 to 4  $\mu\text{g/ml}$ ), the inhibitory effect is inoculum dependent (19, 23, 24, 25a, 27), and the practical significance of this phenomenon for the isolation of *N. gonorrhoeae* remains unclear. In addition, it has been suggested (2, 4, 10-12) that dual plating of endocervical or urethral specimens significantly enhances the isolation rate. To reexamine these issues, we conducted a study in an urban sexually transmitted disease (STD) clinic to compare the rates of isolation of *N. gonorrhoeae* from the urethra or endocervix with modified Thayer-Martin medium (MTM), a vancomycin-free selective medium (VFSM), and noninhibitory chocolate agar (CA).

### MATERIALS AND METHODS

**Patient population.** The Department of Public Health Sexually Transmitted Disease Clinic at Harborview Medical Center has about 27,000 patient visits annually. During the course of this study, all male patients undergoing urethral culture and all women undergoing cervical culture for *N. gonorrhoeae*, whether for diagnosis, screening, or test of cure, were included in the analysis.

**Isolation and identification of *N. gonorrhoeae*.** All media were produced in our laboratory. CA was prepared by supplementing gonococcal agar base (Difco Laboratories, Detroit, Mich.) with final concentrations of 1% (vol/vol)

each of the defined supplement IMX (Medical Media Laboratory, Boring, Oreg.) and hemoglobin. MTM was prepared similarly, with the addition of NVCT inhibitor (Medical Media Laboratory) to achieve final concentrations of 12.5 U of nystatin per ml, 3.0  $\mu\text{g}$  of vancomycin per ml, 7.5  $\mu\text{g}$  of colistin per ml, and 5.0  $\mu\text{g}$  of trimethoprim per ml. VFSM has the same formulation as MTM except for the deletion of vancomycin. Media to be compared were poured into 100-mm diameter sterile biplates, which were stored in sealed containers at 4°C until used, usually within 4 days and always within 14 days of preparation.

Urethral specimens from men were collected by insertion of a urethrogenital calcium alginate-tipped swab (Calgiswab; Inolex, Glenwood, Ill.) 2 to 4 cm beyond the meatus. The swab was used to inoculate both halves of the biplate, and it was rotated 180° between inoculations. Specimens from women were collected by sequential insertion of two polyester-tipped or cotton-tipped swabs into the cervical os, and each swab was used to inoculate half of the biplate. Media were inoculated confluent and were not cross-streaked. Inoculated media were placed in candle extinction jars within 20 min and were incubated at 35°C. After 18 to 24 h, they were transferred to an incubator with a 3 to 6% atmospheric concentration of CO<sub>2</sub> for an additional 24 h. The growth of typical colonies containing gram-negative diplococci giving a positive oxidase reaction provided presumptive identification of *N. gonorrhoeae*.

**Study design.** Biplates containing pairs of media to be compared were provided to the STD clinic staff, who were blinded to the composition of the media in the biplates and were unaware when changes were made; this ensured a random sequence of inoculation of the media being compared. The comparison of CA with MTM was carried out during two time periods: 1 January to 30 April and 1 July to 30 August 1981. From 1 May to 30 June 1981, both halves of the biplates contained MTM. MTM was compared with VFSM from 1 September 1981 to 5 March 1982.

**Statistical method.** The McNemar test for paired alternatives was used to assess statistical significance.

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TABLE 1. Comparative isolation rates of *N. gonorrhoeae* on CA and MTM

Site cultured (no.)	Total <i>N. gonorrhoeae</i> isolations (%)	No. of isolations on the following specific media (% of total isolations):		
		MTM + CA	MTM only	CA only
Urethra (6,236)	868 (13.9)	839 (96.7)	16 (1.8)	13 (1.5)
Cervix (3,032)	326 (10.8)	294 (90.2)	26 (8.0)	6 (1.8)
Total (9,268)	1,194 (12.9)	1,133 (94.9)	42 (3.5)	19 (1.6)

RESULTS

The isolation rates during the two periods of the MTM-CA comparison showed no significant seasonal variation, and the results for the two periods, therefore, were combined (Table 1). *N. gonorrhoeae* was isolated from 1,194 (12.9%) of 9,268 urethral and cervical specimens. The overall sensitivities were 98.4% (1,175 of 1,194) for MTM and 96.4% (1,152 of 1,194) for CA ( $P < 0.01$ ). The difference was due entirely to the results in women, in whom the sensitivities of MTM and CA were 98.2 and 92.0%, respectively ( $P < 0.001$ ).

In the comparison of MTM with VFMSM, 8,779 urethral and cervical cultures were examined (Table 2), with 1,061 (12.1%) isolations of *N. gonorrhoeae*. Among 755 urethral infections in men, 752 (99.6%) were detected with VFMSM, compared with 740 (98.0%) with MTM ( $P < 0.01$ ). By contrast, among 306 cervical infections, MTM gave a slightly higher yield (301 isolations, 98.4%) than VFMSM (293 isolations, 95.8%), but the difference was not statistically significant ( $P = 0.10$ ). The overall sensitivities of the two media were 98.1% (1,041 of 1,061) for MTM and 98.5% (1,045 of 1,061) for VFMSM; the difference is not statistically significant.

Dual inoculation of MTM was performed for 2,412 cervical and urethral cultures (Table 3), from which *N. gonorrhoeae* was isolated from 289 (12.0%). Five isolates (1.7%) were recovered only on one-half of the biplate, representing increased yields of 1.5% for men and 2.4% for women.

DISCUSSION

In this study, *N. gonorrhoeae* was isolated significantly more often on either of the selective media than on CA. The absolute differences were small, however, even for endocervical cultures, and in our laboratory the major advantage of selective over nonselective media is the more efficient use of personnel and other resources, rather than the enhanced isolation rate. The increased yields of the selective media over CA (1.6% for MTM and 1.9% for VFMSM) were comparable to the enhanced isolation rate achieved by dual inocu-

lation of MTM (1.7%). This suggests that the enhanced isolation rate may be due in part to reduction of sampling error, rather than due solely to the superiority of one medium over another.

For urethral infections, VFMSM was superior to MTM, but the difference was small (99.6% versus 98.0% sensitivity;  $P < 0.01$ ). The reverse was true for cervical infections, with a slightly higher yield being observed with MTM than with VFMSM (98.4 versus 95.6% sensitivity), but this difference was of borderline statistical significance ( $P = 0.10$ ). The gonococcal isolates were not saved and their susceptibility to vancomycin was not tested, but none of the 61 other isolates from Seattle were inhibited by  $\leq 4 \mu\text{g}$  of vancomycin per ml (25a). These results confirm that susceptibility of *N. gonorrhoeae* to vancomycin is not a serious issue in our clinic population. Nevertheless, susceptibility of *N. gonorrhoeae* to vancomycin could account for the slightly increased yield of isolates on VFMSM over MTM in men. For cervical infection, the theoretical advantage of VFMSM for isolation of vancomycin-susceptible *N. gonorrhoeae* may be outweighed by less complete suppression of competing flora. However, among the 13 cervical infections detected with MTM but not VFMSM, overt overgrowth on VFMSM occurred in only three instances.

We did not analyze the symptomatic status of the patients included in this study. Vancomycin-susceptible gonococci usually require arginine, hypoxanthine, and uracil for growth ( $\text{Arg}^- \text{Hyx}^- \text{Ura}^-$  auxotype); such strains are more likely to cause asymptomatic infection than are other gonococci (14), at least in men. Therefore, although our results may be applicable to many STD clinics, which selectively attract symptomatic patients (6), they may not be applicable when screening for gonorrhea in other clinical settings, where greater proportions of infected patients may lack symptoms and signs. In this regard, it is perhaps significant that the prevalence of  $\text{Arg}^- \text{Hyx}^- \text{Ura}^-$  gonococci is decreasing in our STD clinic population, from ~50% of strains in the early 1970s (14) to ~10% in 1982 (25a).

In theory, simultaneous use of both selective and nonselective media might maximize the isolation rate of *N. gonorrhoeae*. This would significantly increase costs, both in materials and in personnel time; in our clinic, the 1.6% enhanced yield is too low for such a policy to be cost effective. Inoculation of the same plate of selective medium with two separate swabs (2, 4, 10-12) gives a comparably increased yield at virtually no increase in cost and is supported by our results using dual inoculation on biplates of MTM. For primary isolation of *N. gonorrhoeae* from the cervix, we now routinely inoculate the same plate of MTM with two separate endocervical swabs.

Finally, it is important to stress that the prevalences of various types of *N. gonorrhoeae*, including vancomycin-susceptible strains, vary greatly from one geographic area to

TABLE 2. Comparative isolation rates of *N. gonorrhoeae* on MTM and VFMSM

Site cultured (no.)	Total <i>N. gonorrhoeae</i> isolations (%)	No. of isolations on the following specific media (% of total isolations):		
		MTM + VFMSM	MTM only	VFMSM only
Urethra (5,673)	755 (13.3)	737 (97.6)	3 (0.4)	15 (2.0)
Cervix (3,106)	306 (9.9)	288 (94.1)	13 (4.2) <sup>a</sup>	5 (1.6)
Total (8,779)	1,061 (12.1)	1,025 (96.6)	16 (1.5)	10 (1.9)

<sup>a</sup> In three instances, VFMSM, but not MTM, was overgrown, precluding identification of *N. gonorrhoeae* on VFMSM.

TABLE 3. Isolation of *N. gonorrhoeae* with dual plating on MTM

Site cultured (no.)	Total <i>N. gonorrhoeae</i> isolations (%)	No. of isolations on one or both halves of biplate (% of total):	
		Both halves	Only one half
Urethra (1,597)	206 (14.8)	203 (98.5)	3 (1.5)
Cervix (815)	83 (11.3)	81 (97.6)	2 (2.4)
Total (2,412)	289 (12.0)	284 (98.3)	5 (1.7)

another and among various populations in a given locale (5, 7, 14, 20), and they also fluctuate over time. Thus, uncritical application of published recommendations for the primary isolation of *N. gonorrhoeae* without regard to geographic and demographic differences among the populations studied may give misleading results. When practical, isolation procedures should be based on locally generated data.

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#### LITERATURE CITED

1. Brorson, J. E., I. Holmberg, B. Mygren, and S. Seeberg. 1973. Vancomycin sensitive strains of *Neisseria gonorrhoeae*: a problem for the diagnostic laboratory. *Br. J. Vener. Dis.* **49**:452-453.
2. Chapel, T. A., M. B. Keane, and C. Gatewood. 1976. Combining cervical and rectal cultures for gonorrhea on a single modified Thayer-Martin plate. *Health Lab. Sci.* **13**:190-193.
3. 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.
4. Dans, P. E., and F. Judson. 1975. The establishment of a venereal disease clinic. II. An appraisal of current diagnostic methods in uncomplicated urogenital and rectal gonorrhea. *Sex. Transm. Dis.* **1**:107-112.
5. Handsfield, H. H., J. S. Knapp, P. K. Diehr, and K. K. Holmes. 1980. Correlation of auxotype and penicillin susceptibility of *Neisseria gonorrhoeae* with sexual preference and clinical manifestations of gonorrhea. *Sex. Transm. Dis.* **7**:1-5.
6. Handsfield, H. H., T. O. Lipman, J. P. Harnisch, E. Tronca, and K. K. Holmes. 1974. Asymptomatic gonorrhea in men: diagnosis, natural course, prevalence, and significance. *N. Engl. J. Med.* **290**:117-123.
7. Handsfield, H. H., E. G. Sandström, J. S. Knapp, P. L. Perine, W. L. Whittington, D. E. Sayers, and K. K. Holmes. 1982. Epidemiology of penicillinase-producing *Neisseria gonorrhoeae* infections: analysis by auxotyping and serogrouping. *N. Engl. J. Med.* **306**:950-954.
8. Hipp, S. S., W. D. Lawton, N. C. Chen, and H. A. Gaafar. 1974. Inhibition of *Neisseria gonorrhoeae* by a factor produced by *Candida albicans*. *Appl. Microbiol.* **27**:192-196.
9. Hipp, S. S., W. D. Lawton, M. Savage, and H. A. Gaafar. 1975. Selective interaction of *Neisseria gonorrhoeae* and *Candida albicans* and its possible role in clinical specimens. *J. Clin. Microbiol.* **1**:476-477.
10. Jacobs, N. F., and S. J. Kraus. 1975. Comparison of hemoglobin-free culture media and Thayer-Martin medium for the primary isolation of *Neisseria gonorrhoeae*. *J. Clin. Microbiol.* **1**:401-404.
11. Judson, F. N. 1978. A clinic-based system for monitoring the quality of techniques for the diagnosis of gonorrhea. *Sex. Transm. Dis.* **5**:141-145.
12. Judson, F. N., and B. A. Werness. 1980. Combining cervical and anal-canal specimens for gonorrhea on a single culture plate. *J. Clin. Microbiol.* **12**:216-219.
13. Kay, D., and M. Levison. 1977. *In vitro* inhibition of growth of *Neisseria gonorrhoeae* by genital microorganisms. *Sex. Transm. Dis.* **4**:1-3.
14. Knapp, J. S., C. Thornsberry, G. A. Schoolnik, P. J. Weisner, K. K. Holmes, and the Cooperative Study Group. 1978. Phenotypic and epidemiologic correlates of auxotype in *Neisseria gonorrhoeae*. *J. Infect. Dis.* **138**:160-165.
15. Kraus, S. J. 1979. Culture methods for *Neisseria gonorrhoeae*. *Arch. Androl.* **3**:343-349.
16. Kraus, S. J., R. C. Geller, G. H. Perkins, and D. L. Rhoden. 1976. Interference of *Neisseria gonorrhoeae* growth by other bacterial species. *J. Clin. Microbiol.* **4**:288-295.
17. Martin, J. E., Jr., J. H. Armstrong, and P. B. Smith. 1974. New system for cultivation of *Neisseria gonorrhoeae*. *Appl. Microbiol.* **27**:802-805.
18. Martin, J. E., Jr., and J. S. Lewis. 1977. Anisomycin: improved antimycotic activity in modified Thayer-Martin medium. *Public Health Lab.* **35**:53-62.
19. Mirrett, S., L. B. Reller, and J. S. Knapp. 1981. *Neisseria gonorrhoeae* strains inhibited by vancomycin in selective media and correlation with auxotype. *J. Clin. Microbiol.* **14**:94-99.
20. Noble, R. C., and B. R. Miller. 1980. Auxotypes and antimicrobial susceptibilities of *Neisseria gonorrhoeae* in black and white patients. *Br. J. Vener. Dis.* **56**:26-30.
21. Reyn, A., and M. W. Bentzon. 1972. Comparison of a selective and nonselective medium in the diagnosis of gonorrhea to ascertain the sensitivity of *Neisseria gonorrhoeae* to vancomycin. *Br. J. Vener. Dis.* **48**:363-368.
22. Seth, A. 1970. Use of trimethoprim to prevent overgrowth by *Proteus* in the cultivation of *N. gonorrhoeae*. *Br. J. Vener. Dis.* **46**:201-202.
23. Taylor, E., and I. Phillips. 1979. Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.* **55**:183-185.
24. Thayer, J. 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.
25. Thayer, J. D., and J. E. Martin, Jr. 1966. An improved selective medium for cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Public Health Rep.* **81**:559-562.
- 25a. Totten, P. A., K. K. Holmes, H. H. Handsfield, J. S. Knapp, P. Perine, and S. Falkow. 1983. DNA hybridization technique for the detection of *Neisseria gonorrhoeae* in men with urethritis. *J. Infect. Dis.* **148**:462-471.
26. Wallin, J., and H. Gnarp. 1975. Possible inhibition of *N. gonorrhoeae* by *C. albicans*: a clinical study. *Br. J. Vener. Dis.* **51**:174-175.
27. Windall, J. J., M. M. Hall, J. A. Washington II, T. J. Douglass, and L. A. Weed. 1980. Inhibitory effects of vancomycin on *Neisseria gonorrhoeae* in Thayer-Martin medium. *J. Infect. Dis.* **142**:775.