Primary Isolation of *N. gonorrhoeae* With a New Commercial Medium

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THE DIAGNOSIS of gonorrhea in the female is notoriously difficult and fallible. New and reliable diagnostic methods are urgently needed if we are to control the disease.

A new commercial culture medium for the primary isolation of *Neisseria gonorrhoeae* has been developed by the Baltimore Biological Laboratory (BBL). This medium differs from other conventional commercial chocolate media in that the added enrichment, instead of being blood or yeast extract, is chemically defined (*1*).

A trial of this medium was made using specimens from three groups of patients: females who were conjugal contacts to males with proven gonorrheal urethritis, females who entered the clinic as self-referrals, and males with acute gonorrheal urethritis.

Methods

The efficacy of the medium was judged on cultures of gonococci from urethral and vaginal specimens. Two base media were used: Difco chocolate medium, Difco Laboratories, Detroit, Mich., and BBL chocolate medium, prepared according to the directions of the manufacturers. Bacto supplement B, a yeast extract, was used to enrich the Difco basal medium. The BBL chocolate medium was enriched with the chemically defined supplement "IsoVitaleX" (*1*), which contained vitamin B–12, l-glutamine, adenine, guanine, p-aminobenzoic acid, l-cystine, glucose, diphosphopyridine nucleotide, cocarboxylase, ferric nitrate, thiamine hydrochloride, and cysteine. This chemical supplement was developed by the manufacturer specifically to aid the growth of gonococci.

The cultural diagnosis of gonorrhea has been shown to be greatly improved when the antibiotic supplements polymyxin B-ristocetin (PR) (*2,3*), or vancomycin-colistimethate-nystatin (VCN) (*4*), were added to conventional chocolate media. VCN appears to be more effective for inhibiting saprophytic *Neisseria* and most other bacterial contaminants, while being better tolerated by strains of gonococci, than the PR supplement. Therefore, VCN supplement was added to the two basal media. PR supplement was added to the Difco basal medium as previously described (*4*), and the four media were compared. The final concentration of polymyxin B and ristocetin was

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25 units and 10 micrograms per ml. of medium, respectively; the VCN medium was 3 units vancomycin, 7.5 micrograms sodium colistimethate, and 12.5 units nystatin per ml. of final medium.

Sterile swabs were used to collect exudate from the posterior vaginal fornix and from the male anterior urethra. The inocula were prepared by suspending the exudate in 1 ml. trypticase soy broth (BBL). To obtain a homogenous inoculum, the specimen was mixed by repeated pipetting and 0.1 ml. delivered to each plate of the media tested. The inoculum was spread over the agar surface with an inoculating loop.

The culture plates were examined after 16 hours of incubation in a candle jar at 35° C. Negative plates were incubated an additional 8 hours before being discarded. As is usual at this laboratory (*2–5*), bacterial colonies that showed a positive oxidase reaction and gram-negative diplococci on the selective media were considered to be presumptively positive for *N. gonorrhoeae*. When only single or a few oxidase-positive colonies were present, fluorescent antibody (FA) staining or sugar fermentation measures, or both, were used to rule out saprophytic *Neisseria* and confirm the identification as *N. gonorrhoeae*.

Colonies of gonococci and of contaminating bacteria and yeast were estimated for each of the four media for each specimen. The medium showing the greatest number of colonies of the kind being estimated was assigned an arbitrary value of 4. The other media were compared with it and scored 4, 3, 2, 1, or 0. The scores for each medium were added, and the totals are presented in the table.

Comparison of 4 media for the isolation of *Neisseria gonorrhoeae*

Medium	Total score		Positive cultures	
	Contaminants	Gonococci	Number	Percent determined
Male urethral specimens: [1]				
BBL medium without antibiotics	138	231	89	97.8
Difco medium plus polymyxin B and ristocetin	24	166	86	94.5
Difco medium plus vancomycin, colistimethate, and nystatin	16	173	87	95.6
BBL medium with added vancomycin, colistimethate, and nystatin	20	331	91	100.0
Vaginal specimens: [2]				
BBL medium without antibiotics	306	58	32	54.2
Difco medium plus polymyxin B and ristocetin	76	81	43	72.9
Difco medium plus vancomycin, colistimethate, and nystatin	76	80	47	79.7
BBL medium with added vancomycin, colistimethate, and nystatin	72	158	57	96.6

[1] Of 94 specimens, 91 or 96.8 percent were positive.
[2] Of 102 specimens, 59 or 57.8 percent were positive.

Results

Among the 94 male urethral specimens (see table) there was a reduction of approximately 86 percent in the number of contaminants in the media to which the selective inhibitors PR or VCN were added. More colonies of gonococci were present on the BBL medium, but this produced no significant difference in the number of positive cultures recovered. Ninety-one of the 94 specimens were positive (96.8 percent) and thus, as judged by culture, three patients appeared to have nongonococcal urethritis.

Vaginal specimens, which generally are more heavily contaminated and contain fewer gonococci, constitute a more rigorous test for a medium than specimens from the male urethra. The results obtained with vaginal specimens are shown in the table.

Of the 102 vaginal specimens, 57.8 percent (59 cultures) were positive for gonococci. The addition of VCN to the BBL medium resulted in a 76 percent reduction in contaminants and a 172 percent increase in gonococcal colonies. In contrast to the data for males, there was also a 78 percent increase in the number of vaginal cultures positive for gonococci. Identifiable growth of very minute, punctiform gonococcal colonies, which were almost always present after 16 hours of incubation on the BBL-VCN

selective medium, did not usually appear until 8 hours later on the Difco PR and VCN selective media. No saprophytic *Neisseria* were found in any of the cultures from the specimens.

Specimens from 51.6 percent of 55 self-referred females yielded positive cultures. Of 47 female conjugal contacts to males with proven gonococcal urethritis, specimen cultures were positive in 75 percent. The best results obtained previously (*6*), using FA procedures and nonselective culture media to diagnose vaginal specimens, yielded 48 percent positive results in specimens from females who also were named as contacts to males with gonorrhea.

Summary and Conclusions

A new commercial medium supplemented with a chemically defined enrichment has been tested for primary isolation of gonococci from vaginal and male urethral specimens.

When this medium was made selective for *Neisseria gonorrhoeae* by adding the antimicrobial inhibitors vancomycin, colistimethate, and nystatin, bacterial and yeast contaminants were suppressed to the same extent as in media containing polymyxin B and ristocetin (PR). Recovery of gonococci was as good or better than on the previously recommended PR medium.

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Reducing the Cost of Artificial Kidneys

The National Institute of Arthritis and Metabolic Diseases, Public Health Service, has launched a program to find ways to simplify and reduce the cost of artificial kidney treatment for patients with chronic kidney failure. While some patients in chronic kidney failure are being kept alive through repeated treatment with the artificial kidneys currently in use, most candidates for such treatment are not receiving the benefits because of the extremely high cost and complexity of treatments.

Twenty-four research and development contracts totaling approximately $1.8 million have been awarded, under the artificial kidney program of the Institute, toward developing a simpler, more effective, more efficient, and more economical artificial kidney than is now available.

The contracts are for projects ranging from improvement of membranes, shunts, and blood vessel connections to development of an artificial kidney small enough to be worn by the patient.

National Center for Urban and Industrial Health

The Public Health Service has created a National Center for Urban and Industrial Health in Cincinnati, Ohio, as part of the recently established Bureau of Disease Prevention and Environmental Control. On January 1, 1967, the new Bureau superseded the Bureau of State Services in the reorganization of the Public Health Service.

The center incorporates six programs which the Service will transfer from Washington, D.C., to Cincinnati and, excluding air pollution and radiological health work, consolidates all the Service's activities in the Cincinnati area. (A National Center for Air Pollution Control and a National Center for Radiological Health are separate organizational components in the Bureau.)

The National Center for Urban and Industrial Health is concerned with problems associated with accidents, occupations, water supplies, housing, solid waste disposal or salvage, food protection, general sanitation, metropolitan planning, and living in arctic or subarctic areas. In discharging its responsibilities, the center conducts research and development, surveillance activities, demonstration and pilot projects, and technical training.

It also renders technical assistance to official and other agencies, develops criteria and codes for use in alleviating health problems, administers grants, and provides consultants for international health programs. In addition, the center certifies interstate milk shippers, administers interstate carrier and quarantine regulations, and effects shellfish sanitation agreements with various States, Canada, and Japan. Four specialized groups aiding the operating programs are the Training Program, Epidemiology Program, Development and Control Branch, and Standards and Codes Branch.

The center is directed by Jerome H. Svore, and Dr. Paul W. Kabler is deputy director. Clarence L. Cade, the center's executive officer, heads its Office of Administrative Management.

The Environmental Sanitation Program, encompassing the former Division of Environmental Engineering and Food Protection, focuses on the prevention and control of health hazards in urban and recreational environments. It fosters housing hygiene, prevention of overcrowding, food protection, and area sanitation. Richard Vaughn is chief of this program.

The Occupational Health Program, which embodies the former Division of Occupational Health, conducts a national effort to prevent and control diseases and injuries incurred by persons at work. The program, with Dr. Murray C. Brown as its chief, operates the Appalachian Laboratory for Occupational Respiratory Diseases, Morgantown, W. Va.

The Solid Wastes Program, headed by Leo Weaver, is mounting a nationwide campaign to protect the public health and welfare from the hazards of solid waste by promoting improvement of solid waste disposal and salvage techniques. The program includes activities of the former Office of Solid Wastes, which operated in Washington with some staff in Cincinnati.

The Water Supply and Sea Resources Program deals with public health and medical aspects of the uses of fresh and salt water and the plant and animal populations of the principal bodies of water. This program, concerned with the health aspects of water pollution control and with shellfish sanitation, certifies water supplies used by interstate carriers and operates shellfish laboratories at Narragansett, R.I., Dauphin Island, Ala., and Gig Harbor, Wash. The program chief is Frederick S. Kent.

The Injury Control Program conducts numerous activities to reduce accidental death and injury. Dr. Richard E. Marland is chief of this program.

The Arctic Health Program, which has Dr. Edward Scott as its chief, is directed toward abating and preventing diseases affecting persons living in arctic and subarctic communities with a view of aiding in the development of those areas. The program operates the Arctic Health Research Center in Anchorage, Alaska.

The Training Program is headed by James P. Sheehy, and offers intensive training to professional persons working in the fields of urban and industrial health.