Technical Reports

Transgrow, a Medium for Transport and Growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*

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FAILURE to diagnose gonorrhea in women who harbor asymptomatic infection is a significant handicap in the control of gonorrhea. This handicap would be partially alleviated if a culture method could be made readily available to all physicians and clinics. Similar considerations apply to detection of nasopharyngeal carriers of meningococci in confined populations.

A selective medium for the cultivation of gonococci and meningococci was reported by Thayer and Martin in 1964 (*1*). An improved medium was described by the same authors in 1966 (*2*). The Thayer-Martin selective medium is recommended for primary isolation of the gonococcus and meningococcus, especially from sites where these organisms are outnumbered by the more rapid-growing natural bacterial flora. Overgrowth by gram-positive and other gram-negative bacteria and yeast is prevented in most cases because the medium contains 3 units of vancomycin, 7.5 micrograms of colistimethate sodium, and 12.5 units nystatin per milliliter. Although this selective medium has been available since 1964, its use, unfortunately, is limited to large hospitals and health centers closely associated with laboratory facilities.

The average practitioner is handicapped in his diagnosis of asymptomatic gonorrhea by the hazards of transportation of suspected specimens to a distant laboratory. At present the most widely used tools for transporting secretions suspected of containing gonococci are the medium of Stuart and coworkers (*3*), a modified version of which was described by Amies in 1967 (*4*), and culture plates in candle jars (*5*).

The procedure used by both Stuart and Amies is designed to eliminate oxidation as a cause of death of the gonococcus while the non-nutrient, non-toxic menstruum supresses growth of the gonococcus and other micro-organisms. Although these media seem to be effective in maintaining viability of organisms during transport times of less than 24 hours, their usefulness is somewhat restricted for longer transport periods. The candle jar may be used for transporting specimens, but this method is cumbersome and therefore less practical.

An ideal transport medium for secretions suspected of containing gonococci and meningococci should be selective for these pathogens, have a prolonged shelf life, and be of rigid composition for mailing. In addition, it should preserve the viability of the organisms for more than 24 hours at ambient temperatures. Eliminating the required transfer from the non-nutrient transport medium to an isolation plate by combining the transporting vehicle with a culture medium would also be desirable.

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Modifications of the Thayer-Martin medium incorporating some of these characteristics are described in this report.

Materials and Methods

All media and supplements used throughout this study were produced by Difco and Bioquest.

The stock cultures of bacteria used were from our collection. Suspensions of stock organisms were prepared by washing off the 20-hour growth with trypticase soy broth (TSB) and diluting to give a 45 percent transmittance of light in a spectrophotometer (Bausch and Lomb spectronic 20, wavelength 530). The stock suspensions were diluted to 10^{-3} for testing.

Secretions from 163 patients suspected of gonorrheal infection were obtained from William E. Hardegree, Fulton County Health Department, Atlanta, Ga., and from A. D. Farmer of the Norfolk (Va.) City Health Department. Each specimen was taken by cotton swab and placed in 1 ml. of sterile TSB. The swabs were rotated and the excess fluid expressed against the side of the tube. To promote homogeneity of the suspension, the specimen was mixed in a Vortex shaker. Two swabs were placed in the mixture, and one swab was used to inoculate each of the media tested.

Twenty-five recent isolates of meningococci were supplied by Dr. W. E. Dunkelberg of the Third U.S. Army Laboratory, Fort McPherson, Ga.

The experimental medium for transport and growth (Transgrow) of *Neisseria gonorrhoeae* and *Neisseria meningitidis* was prepared as follows:

Mixture 1

GC medium base agar, 36 gm.
Agar, 10 gm.
Distilled water, 500 ml.

Mixture 2

Hemoglobin, 10 gm.
Distilled water, 500 ml.

After the two mixtures were autoclaved (121° C., 15 minutes) they were allowed to cool to 56°C. Then to mixture 1 was added 10 ml. of an enrichment—Supplement B (Difco) or Isovitalex (Bioquest), 10 ml. of VCN antibiotic supplement (2), and 6 ml. of a sterile 25 percent dextrose solution. After thorough mixing, mixture 1 was combined with mixture 2.

Eight ml. of the liquid Transgrow medium was then poured into a horizontally positioned 1-ounce prescription bottle (Brockway Glass Company). A rubber-lined screwcap was loosely applied, and the medium was allowed to cool with the bottle remaining horizontal until solidification occurred. A CO_2 atmosphere was introduced into the bottle by placing a group of bottles containing solidified medium upright in a vacuum jar, partially exhausting the air with a vacuum pump, and refilling the chamber with a 10 percent CO_2-90 percent filtered air mixture (commercially obtained) until the chamber returned to atmospheric pressure. Screwcaps were then tightly fastened.

The medium was inoculated by briefly removing the screwcap and rolling the swab from side to side over the surface of the agar. During the inoculation procedure, the mouth of the bottle was kept elevated to minimize the loss of CO_2. The caps were then replaced and tightened.

Following inoculation, the bottles were placed in a urethane container (Phillips-Foscue Company) and mailed from the Norfolk, Va., laboratory to the Venereal Disease Research Laboratory in Atlanta. Transit time varied from 48–96 hours. Upon receipt, the bottles were incubated for 16 hours at 35°C. Oxidase test reagents were applied to suspected colonies. Samples of oxidase-positive colonies were taken for gram-staining and subculturing for analysis by sugar fermentation. *N. gonorrhoeae* was said to be present if typical oxidase-positive colonies contained gram-negative diplococci and fermented glucose but not maltose.

Results

The ability of Transgrow to suppress the growth of saprophytic *Neisseria,* while allowing the growth of pathogenic *Neisseria* in a manner comparable to that of Thayer-Martin medium, was assessed.

Table 1.—Selective growth of *Neisseria* species on three media

Organism	Number of strains	Chocolate agar	Thayer-Martin	Transgrow
Neisseria gonorrhoeae	45	45	45	45
Neisseria meningitidis A	7	7	7	7
Neisseria meningitidis B	3	3	3	3
Neisseria meningitidis C	1	1	1	1
Neisseria meningitidis (Dunkelberg)	25	25	25	25
Neisseria catarrhalis	8	8	2 ±	2 ±
Neisseria flava	2	2	----	----
Neisseria perflava	5	5	----	----
Neisseria subflava	1	1	----	----
Neisseria sicca	3	3	----	----
Neisseria flavescens	1	1	----	----

Table 2.—Summary of results on 163 specimens from 124 male and female patients with suspected gonococcal infection

Sites	Number of specimens	Number positive	
		Thayer-Martin [1]	Transgrow [2]
Male urethra	71	63	63
Female cervix	61	40	39
Female rectum	31	8	8
Total	163	111	110

[1] Immediate incubation at 35.5° C.
[2] Elapsed time of 48–96 hours from inoculation to incubation at 35.5° C.

The study was performed by using a selection of laboratory strains. Table 1 shows that Transgrow and Thayer-Martin medium gave comparable suppression of these saprophytes.

Forty-five cultures of gonococci and the 25 isolates of meningococci were inoculated into bottles of Transgrow. These were mailed from our laboratory to our laboratory and received 48 hours later. Upon arrival they were incubated at 35.5° C. for 16–24 hours. All isolates were viable.

The comparison of Transgrow medium with Thayer-Martin medium is shown for suspected gonococcal secretions in table 2. We wish to emphasize that the specimens inoculated on Transgrow medium were in transit 48–96 hours at ambient temperature before they were received by the laboratory and incubated. Of the 163 specimens, results for the Transgrow medium and the Thayer-Martin medium agreed on 162. For one female cervical specimen the Transgrow culture was negative, whereas the Thayer-Martin culture was positive.

Discussion

The Transgrow medium differs in several respects from Thayer-Martin medium although it generally represents a further evolution of it. The principal modifications are:

1. Increased percentage of agar (from 1 percent to 2 percent)
2. Increased percentage of dextrose (from 0.1 percent to 0.25 percent)
3. Use of surface area obtained in a flat bottle
4. Use of controlled atmosphere of 10 percent CO_2-90 percent air

Increasing the agar concentration resulted in a rather rigid, mailable medium. The 2 percent concentration was chosen as the best balance between rigidity and adequate growth of gonococcal colonies. Greater amounts of agar resulted in reduced colony size after 20 hours' incubation. The dextrose concentration was increased on the basis that a few isolates of *N. gonorrhoeae* seemed to require the additional dextrose to achieve good growth. A flat bottle rather than a tube was selected because it has a larger surface area and is more convenient for the laboratorian. The use of the 10 percent CO_2-90 percent air mixture is in accord with Ferguson's demonstration that this gives growth equivalent to the candle extinction procedure (*6*).

Recently, Robinson and co-workers (*7*) reported favorable results using slants of Thayer-Martin medium with VCN to transport female specimens. Their study differs from ours in that specimens were incubated immediately in a 5–10 percent CO_2 atmosphere at 35–37° C., and were kept there overnight before mailing. Also, upon arrival at the laboratory bacteria growth was taken from the slant and streaked on the surface of a plate of Thayer-Martin medium. When the Robinson results were compared with the delayed fluorescent antibody method, the slants maintained viability on 61 percent of the transported specimens.

Although the media suggested by Stuart and co-workers and modified by Amies is presently used by many laboratories, they have two disadvantages. First, there appears to be a significant loss of organisms in the non-nutrient environment after 24 hours, especially in the case of gonorrhea cultures from females. Second, upon arrival in the laboratory it is necessary to transfer the specimens to a culture plate. Transgrow effectively bypasses these problems since the transport medium and culture medium are one and the same. Transfer of organisms to nutrient medium on arrival at the laboratory is therefore unnecessary. Furthermore, our data show survival after 48–96 hours at ambient temperature. Preliminary field trials of Transgrow medium are underway, and early data from these are encouraging, according to personal communications from Dr. Dunkelberg and A. D. Farmer.

When inoculated Transgrow is incubated 16–18 hours at 34–37° C. before the bottle is mailed, growth will occur before shipment. This growth survives prolonged transport and is ready for definitive examination immediately upon arrival at the laboratory. For example, 25 gonococcal isolates obtained in Thailand were inoculated there on Transgrow medium and incubated at 35.5°C. before mailing. Upon receipt in our laboratory, after 6 days in the mail, 24 of the 25 isolates were viable.

In summary, Transgrow appears to have potential as a valuable tool for transportation of suspected meningococcal and gonococcal specimens.

If field evaluations confirm the findings presented here, the availability of this medium will enable central laboratories to make routine gonococcal culturing and screening for meningococcal carriers available to all physicians and public health workers.

ADDENDUM

Early reports from field evaluations using Transgrow medium for detection of meningococci in nasopharyngeal specimens and gonococci in cervical specimens, in general, support the findings presented here. However, the effectiveness of the medium for detecting rectal gonorrhea has been hampered to some extent by the problem of overgrowth, usually by *Proteus* "spreaders," on rectal specimens.

Recently, Seth (*8*) and Dr. I. Phillips of the London Hospital, London, England (personal communication, June 1970) reported that the addition of trimethoprim lactate (Burroughs Wellcome & Company) to a gonococcal culture medium containing the Thayer-Martin inhibitors (*2*) suppressed the growth of *Proteus* species without interfering with the growth of gonococci. This significant contribution has been examined with regard to Transgrow medium.

Fifty isolates of *Neisseria gonorrhoeae* grown on Thayer-Martin medium were each diluted to 10^{-3} in trypticase soy broth. A 3-mm. loopful of each suspension was inoculated onto the surface of three separate preparations of Transgrow medium containing 5, 7.5, or 10 μg. per ml. of trimethoprim lactate. Slight inhibition of gonococcal growth of several strains was observed at 10 μg. per ml. when a comparison was made with Transgrow medium that contained no trimethoprim. No inhibition was observed at the 7.5 and 5 μg. per ml. concentrations.

Of 41 recent isolates of *Proteus* species tested, spreading of 39 strains was completely suppressed at the three concentrations studied.

Based on the available data, the Transgrow medium will incorporate 5 μg. per ml. of trimethoprim. This is accomplished by combining trimethoprim and the Thayer-Martin inhibitors before they are added to the medium.

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The private practitioner is handicapped in his diagnosis of asymptomatic gonorrhea by the hazards of transportation of suspected specimens to a distant laboratory. An experimental medium for transport and growth (Transgrow) of *Neisseria gonorrhoeae* and *Neisseria meningitidis* has been developed.

A comparison was made of Transgrow medium with Thayer-Martin medium on 163 suspected gonococcal specimens. The specimens inoculated on Transgrow medium were in transit 48–96 hours at ambient temperature before they were received by the laboratory and incubated at 35.5° C., whereas the specimens inoculated on Thayer-Martin medium were incubated immediately at 35.5° C. Results for both mediums agreed on 162 specimens.

Field trials are underway and early data from these are encouraging. Transgrow appears to have potential as a valuable tool for transportation of suspected meningococcal and gonococcal specimens. If field evaluations confirm the findings presented, the availability of Transgrow medium will enable central laboratories to make routine gonococcal culturing and screening for meningococcal carriers available to all physicians and public health workers.

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