Comparison of Transgrow and T-M Media for *Neisseria meningitidis* Surveys

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THE Third U.S. Army Medical Laboratory has a continuing requirement to survey the relative frequency of the carrier rate for *Neisseria meningitidis* among recruits at training centers. This requirement provided an opportunity to study the suitability of a new medium, Transgrow, to transport *N. meningitidis* contained in throat transudates through the mails. It also allowed us to evaluate Transgrow's selectivity as a medium to grow organisms after incubation at the laboratory.

Materials and Methods

Media. Parallel tests were made of Thayer-Martin (T-M) medium dispensed in petri plates and bottled Transgrow medium. The T-M medium was a Mueller-Hinton base with 5 percent chocolated sheep blood and VCN (vancomycin-colistimethate-nystatin) antibiotics. Formulation of Transgrow, a modified T-M medium containing increased concentrations of agar and dextrose, was described by Martin and Lester (*1*). Transgrow was dispensed at 6 ml. of medium per Mycoflask.

Mycoflask units were gassed for 30 minutes with a commercially purchased mixture of 10 percent CO_2 and 90 percent air in a large glass vessel. Screwcaps were then secured firmly to retain the gas in the containers. Transgrow medium was supplied by the Neisseria Unit, Venereal Disease Research Laboratory, Center for Disease Control, Atlanta, Ga.

Operations. The training centers used in the study were those routinely surveyed by this laboratory for meningococcal carrier rates. They were Fort Jackson, S.C., 243 air miles from Atlanta; Fort Campbell, Ky., 339 air miles; and Fort Bragg, N.C., 406 air miles. Recruits selected for surveys were in the sixth week of basic training, and they had not received the meningococcus vaccine currently being evaluated by others.

Collections and mailing. Tongue blades and sterile plain cotton-tipped swabs were used to collect throat specimens. Two swabs held side by side were used for each specimen. One swab was rolled and streaked over the surface of the T-M plate medium while the other was used to inoculate bottled Transgrow medium in the same manner. Screwcaps were replaced tightly on the bottles to retain the gas.

The Transgrow bottles were packed in foam-lined paper boxes, 20 bottles per box, and mailed to Atlanta. They were received at the

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laboratory in 24 to 48 hours, unpacked, and incubated for 18 hours at 36°C., with agar surfaces in a vertical position. Candle extinction was not necessary because of the gassing previously described. T-M plates were hand carried and flown to Atlanta. The medium was incubated within 4 to 5 hours after streaking and incubation was for 18 hours under candle extinction. According to unpublished data of J. R. Creitz and co-workers, there is no reduction in positive rates if T-M plates are incubated within 8 to 10 hours after streaking.

Bacteriology. Plates and bottles were examined after the incubation periods. Plates and bottles showing no growth after 18 hours' incubation were reincubated an additional 24 hours under the same conditions. Cultures showing moderate to heavy growth of colonies morphologically like *N. meningitidis* were tested by covering representative colonies with 1 percent aqueous solution of dimethyl-p-phenylene-diamine HCl. Oxidase positive colonies were serotyped by slide agglutination tests with type specific meningococcal antiserums. Van Peenen and co-workers demonstrated that identification of *N. meningitidis* is greatly simplified when good growth is obtained from throat swabs cultured on T-M medium (*2*).

Specimens yielding scant numbers of suspect colonies were subcultured to T-M plates and were retested. Organisms which agglutinated in 0.9 percent saline solution or failed to agglutinate in meningococcal antiserums were inoculated into 1 percent carbohydrate-cystine trypticase agar media and stained with gram reagents. Those that had gram-negative diplococci and fermented only dextrose and maltose were recorded as nontypable *N. meningitidis*.

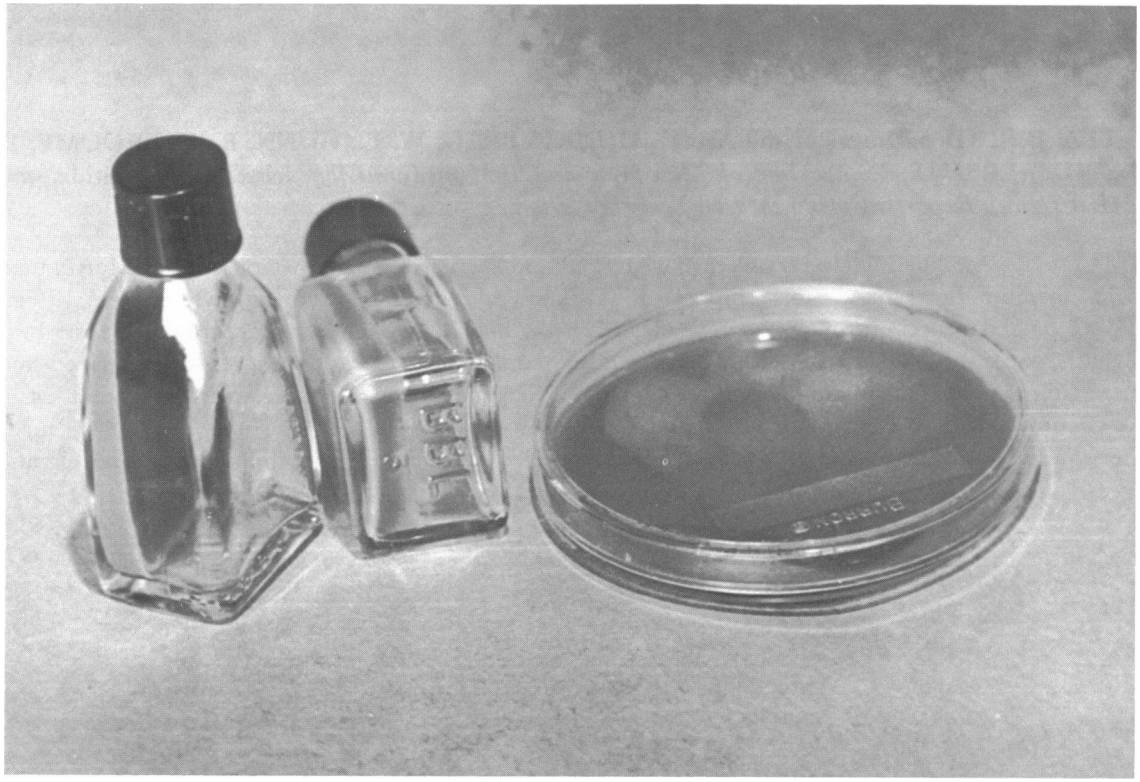
Results

The results of parallel tests of specimens from 634 recruits when T-M and Transgrow media were compared follow.

Thayer-Martin	*Transgrow*	*Total*
Positive _____	Positive ____	169
Positive _____	Negative ___	35
Negative _____	Positive ____	22
Negative _____	Negative ___	408

A total of 204 specimens grown on Thayer-Martin medium were positive, as were 191 grown on Transgrow. The Transgrow medium, used as described in this paper, was 93 percent as effective as the T-M plate medium. The difference was not significant.

There was no apparent difference in positive rates between



Two Transgrow units in Mycoflasks, manufactured by Baltimore Biological Laboratories (left), and plate containing Thayer-Martin medium (right)

Transgrow units requiring 24 to 48 hours' transit time to the laboratory. Organisms serotested from Transgrow medium agreed in serotype with organisms from T-M medium in every instance but one.

Discussion

In our experience, mailed Transgrow medium was 93 percent as effective as hand-carried air-shipped T-M plate medium for selective recovery of meningococci. Only two bottles were broken in shipment, and this breakage was due to inadequate packing. None were broken when bottles were individually wrapped with batting or paper hand towels.

Bottles mailed to the laboratory did not show growth of meningococci upon receipt. Growth occurred during the 18-hour incubation period at 36°C. In most cases, the volume of growth was comparable on both media. Furthermore, selective growth of meningococci was the same on both T-M and Transgrow. Spreading *Proteus* species appeared on less than 5 percent of both media.

Meningococci on Transgrow showed a delayed positive oxidase test. The reaction can be obtained by applying the oxidase reagent and then allowing the bottles to rest with caps off, or stirring the colonies with a sterile loop, or forcing air into the bottle. We prefer to use Kovacs' oxidase test method on colonies from Transgrow (*3*). Reactions of organisms applied to filter paper saturated with oxidase reagent were rapid and distinct.

This study was conducted when ambient temperatures were mild. We have had no experience with Transgrow when mailed during cold weather.

REFERENCES

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(*2*) Van Peenen, P. F. D., Suiter, L. E., Mandel, A. D., and Mitchell, M. S.: Field evaluation of Thayer-Martin medium for identification of meningococcus carriers. Amer J Epidem 82: 329–333 (1965).

(*3*) Kovacs, N.: Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) 178: 703 (1965).

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Transgrow, a modified Thayer-Martin medium, was used to transport meningococci through time and space and to cultivate the organisms upon incubation. Parallel studies of meningococcal carrier rates among Army recruits were made using hand-carried, air-shipped T-M plates and mailed bottles of Transgrow media.

The results of parallel tests of specimens from 634 recruits when T-M and Transgrow media were compared follow: 204 specimens grown on Thayer-Martin medium were positive, as were 191 grown on Transgrow. Transgrow medium, used as described in this paper, was 93 percent as effective as the T-M plate medium. The difference was not significant.