USE OF VANCOMYCIN, COLISTIMETHATE, NYSTATIN MEDIUM TO TRANSPORT GONOCOCCAL SPECIMENS

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THERE are problems associated with the diagnosis of gonorrhea in the asymptomatic female. For many years, the sulfonamides have had little effect in inhibiting the gonococcus, and surveillance studies indicate ever-increasing resistance to penicillin, streptomycin, and oxytetracycline (*1*, *2*). For this reason, it is expedient to obtain accurate laboratory diagnosis rapidly in primary cases and to indicate later whether treatment with antibiotics has been successful.

The conventional smear stained by Gram's method is of value in early infections when typical intracellular diplococci may be present in material taken from genital orifices. These organisms are more likely to be found in the male than in the female by this direct examination. As the infection advances or in chronic cases where few organisms are present, the value of the smear decreases (*3*).

Because the genitourinary tract harbors staphylococci, streptococci, various rod types, and *Mimeae* or other species of *Neisseria* which resemble the gonococcus, information obtained from smears may not be reliable. When the examination is not performed in a venereal disease or a reference laboratory, uncertainty may be compounded because many diagnostic laboratories receive relatively few requests to identify *Neisseria gonorrhoeae* as compared with the number of such specimens submitted to specialized laboratories.

In an effort to broaden the examination, many workers also culture various exudates on chocolate agar which well supports the growth of the gonococcus. But due to overgrowth, the presence of numerous bacteria that show positive oxidase reactions, and variations in colonial morphology among gonococcal varieties, correct identification of organisms may be difficult (*4*, *5*). In recent years immunofluorescent techniques for identifying gonococci have been used successfully (*6*). Thayer and Martin (*7*) have developed a selective medium (vancomycin, colistimethate, nystatin medium) for the isolation and cultivation of *N. gonorrhoeae*. This medium, hereafter referred to as VCN, was prepared in the Ohio Department of Health's laboratory and was used in this study.

The purpose of this paper is to report our experience using VCN as a transport medium and to demonstrate the higher percentage of positive results obtained by combining immunofluorescence with culture procedures. This study began in March 1966 and ended in January 1968. The procedure described in the study is now being followed routinely at the Ohio Department of Health laboratories.

Materials and Methods

The medium was prepared from commercially available dehydrated gonococcus medium base (*A*) and to the base was added IsoVitaleX

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enrichment (*A*), and VCN inhibitor (vancomycin, colistimethate, nystatin) (*A*). Six milliliters of the medium were tubed, slanted, and, along with a ringed fluoroslide (*B*), mailed to participating laboratories.

Recipients of the tubes and fluoroslides were instructed to inoculate the VCN slant with a swab containing clinical material, taking care not to break the surface of the medium. Using the same swab, the fluoroslide was to be smeared (this would be the direct FA), and the swab returned to the slant. The media then had to be incubated in a 5–10 percent CO_2 atmosphere at 35–37° C., and the following day both the slant and the slide were to be mailed.

If the organisms had not grown sufficiently when the slants were received in the laboratory, the slants were incubated an additional 18–24 hours. Otherwise, the swab was removed and used to prepare a smear for Gram's staining and a fluoroslide for the indirect FA examination.

Growth from the slant was restreaked onto a VCN plate. After a 14-hour incubation period in approximately 10 percent CO_2, plates with colonies showing typical microscopic and macroscopic morphology and positive oxidase reactions were restreaked for purification. Single colonies were then picked individually to separate tubes of cystine trypticase agar (CTA) containing 1 percent glucose, sucrose, and maltose.

Both the direct and indirect slides were fixed in 3 percent formalin in saline and stained for fluorescent antibody examination using the method of Thayer and co-workers (*3*). The smears were examined with an ultraviolet microscope equipped with BG–12 and OG–1 filters.

Fermentation reactions were considered positive when a color change occurred in the CTA tubes and when Gram's stained smears indicated no contaminating organisms. Organisms fermenting glucose but not sucrose or maltose were reported as *N. gonorrhoeae*.

Results of 4 procedures on each of 768 specimens tested for gonococci

Test	Positive results	
	Number	Percent
Direct FA	90	11.8
Delayed FA	163	21.2
Delayed Gram's stained	165	21.5
Culture	100	13.0

Results and Discussion

A total of 768 specimens was submitted for both the direct FA examination and for culture. The table shows 11.8 percent of the specimens were positive by the direct examination. This figure nearly doubled with the indirect FA and the indirect Gram's stain. The 13 percent recovery from the cultures indicated specimens containing viable organisms which could be confirmed by fermentation reactions. This figure is slightly higher than those found by direct examination. Probably some growth occurred during shipment, but the organisms were nonviable at subculture.

Only 46, or 6 percent, of the 768 specimens submitted were positive by all tests: direct FA, delayed FA, and delayed Gram's stained smear tests and subculture. In addition to the 768 specimens, 193 slides were submitted for direct FA test only. Twenty-seven, or 14.1 percent, of these slides were positive. There was no way to determine if a higher percentage would have been positive by other tests.

The FA examination was considered positive when organisms showing typical diplococcal morphology fluoresced 3+ to 4+. Nonspecific staining was minimized by adding equal parts of human and rabbit serum to the conjugate (*3*).

Most specimens were received by mail within 2 days from physicians or other laboratories outside Columbus and usually were not accompanied by a history. Specimens from local physicians were delivered by messenger. Therefore it could not be determined which patients were clinically positive for gonorrhea. All specimens were taken from women, and some specimens were resubmitted 7–10 days after a positive report had been mailed, 3.9 percent of the total specimens were resubmitted. Positive results from the second specimen could have occurred either as a result of resistant gonococci or because of reinfection.

Earlier studies indicate that dead gonococci from the cervix or urethra may carry over to the delayed FA, yielding positive results although the culture remains negative. Our studies yielded positive results by direct exami-

nation for 17, or 2.2 percent, of the organisms which failed to grow in culture. Also, because histories were lacking, no interpretation of these figures was attempted.

In the past, mail transport of materials suspected of containing gonococci has been difficult. Although various other mediums have been used to transport specimens with some degree of success, we have found the VCN medium to be adequate. The slants and slides are readily made into a kit that can be handled easily and stored for short periods.

Overgrowth has not been a significant factor, and we have recovered the gonococcus even though some cultures were not incubated before mailing. Supplying the necessary moisture to prevent drying, the proper nutrients, and inhibitors against overgrowth, VCN serves as both transport and primary culture medium.

The materials used in the procedures described in the paper are readily available. They make accessible to all physicians adequate facilities with well-trained personnel for the detection of *N. gonorrhoeae*.

Summary

Vancomycin, colistimethate, nystatin medium served as primary culture and transport medium for 768 gonococcal specimens mailed to the Ohio Department of Health's division of public health laboratories for examination. Using the fluorescent antibody (FA) test, 193 direct smears were examined also.

Only 46, or 6 percent, of the 768 specimens were positive by all tests: the direct FA, delayed FA, and delayed Gram's stained smear tests and subculture. Twenty-seven, or 14.1 percent, of the slides submitted for direct FA examination were positive.

Cultural procedures combined with fluorescent antibody techniques on 768 specimens produced a higher percentage of positive results than would have been derived from either the direct Gram's stained smear, direct FA examination, or by cultures alone. Fermentation studies were used to confirm morphologically positive cultures.

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SUPPLY REFERENCES

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