

Primary Isolation of *Neisseria gonorrhoeae* on Hemoglobin-Free New York City Medium

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Received 2 February 1981/Accepted 8 April 1981

New York City medium and New York City medium without hemoglobin were comparatively evaluated for their ability to support the growth of *Neisseria gonorrhoeae* isolated from 1,010 clinical specimens. Although hemoglobin in the form of lysed horse erythrocytes stimulated gonococcal growth, the absence of this component from New York City medium did not have a detrimental effect on the recovery of gonococci isolated from clinical specimens. Both media were comparable in their ability to cultivate gonococci from clinical material, with a total of 187 gonococcal isolates being recovered on each of the media. The results of this study showed that the preparation of New York City medium can be facilitated and that its cost can perhaps be reduced by the elimination of the hemoglobin component from the formulation without adverse effect on the recovery of *N. gonorrhoeae* isolated from clinical specimens.

In 1973, Faur and her associates (3, 4, 7) developed New York City (NYC) medium, which was reported to support the growth of *Neisseria gonorrhoeae* and urogenital mycoplasmas (8). Clinical evaluation of the original or modified versions of NYC medium have shown it to be comparable (9, 18, 19), and in some reports superior (7, 20), to various formulations of Thayer-Martin medium for the recovery of *N. gonorrhoeae* isolated from clinical material.

Because several investigators (1, 2, 14, 16) have reported a 10% incidence of vancomycin-sensitive strains of *N. gonorrhoeae*, an improved NYC medium was subsequently developed by Faur and her colleagues (6) by reducing the concentration of vancomycin in the medium. As reported, the use of this NYC medium resulted in enhanced recovery of *N. gonorrhoeae* isolated from clinical specimens as compared with the original NYC medium. Furthermore, in a recent evaluation comparing NYC medium with Martin-Lewis medium (11), we showed that the use of NYC medium results in a 13% overall increased recovery of gonococci isolated from clinical material.

Although both formulations of NYC medium have been shown to enhance the recovery of gonococci isolated from clinical specimens, widespread use of either of these media has been limited because of their complexity and cost of preparation. Because several studies (12, 13, 15,

17) have shown that hemoglobin is not a necessary enrichment in Thayer-Martin medium as well as in other media for supporting the growth of gonococci isolated from clinical specimens, we reasoned that the preparation of NYC medium could be facilitated and that its cost could possibly be reduced if the hemoglobin source (lysed horse erythrocytes) could be eliminated from the formulation. The purpose of this study was to comparatively evaluate NYC medium and NYC medium without hemoglobin (NYC-Hb medium) for the recovery of *N. gonorrhoeae* isolated from clinical specimens.

MATERIALS AND METHODS

NYC medium was prepared as originally described by Faur and her associates (3, 4, 7) except that the vancomycin concentration was reduced to 2 $\mu\text{g/ml}$ (8). NYC-Hb medium was prepared in a manner similar to that for NYC medium except that an equal volume of distilled water was substituted for a 3% solution of lysed horse erythrocytes. Both media were poured into JEMBEC plates, and each batch was performance tested before use for its ability to support the growth of *N. gonorrhoeae* as well as to inhibit the growth of *Escherichia coli* and enterococci. All media were stored at 4°C and warmed to room temperature before use.

A total of 1,010 clinical specimens were collected from walk-in patients attending the Sexually Transmitted Disease Clinic at the Onondaga County Department of Health, Syracuse, N.Y. Clinical samples were collected by trained nurses from the urethra, cervix, pharynx, or rectum. Urethral specimens were collected with calcium alginate-tipped applicators

(Becton, Dickinson & Co., Rutherford, N.J.), and specimens from the other anatomical sites were collected with cotton-tipped swabs.

Swab specimens that were collected for culture were placed in tubes containing 0.7 ml of a sterile 0.4% gelatin solution in distilled water. The swab was twirled in the solution to elute the clinical material, rimmed against the side of the tube to express the excess fluid, and then discarded. Each of the two test media were inoculated with a separate applicator that was dipped into the sample solution. The swab was rolled slowly over the surface of the test medium in a large "Z" pattern to maximize transfer of the inoculum to the agar surface. A carbon dioxide-generating tablet was placed in the well of each chamber, and each plate was sealed in a plastic "zip-lock" environmental pouch and immediately placed in a 35°C incubator.

All JEMBEC plates were incubated for 72 h and examined daily for the appearance of microbial growth. Bacterial isolates were identified as *N. gonorrhoeae* on the basis of colonial morphology, Gram stain, oxidase reaction, and carbohydrate utilization pattern in cystine-Trypticase (BBL Microbiology Systems, Cockeysville, Md.) agar media.

RESULTS

The recovery rates on NYC and NYC-Hb media of *N. gonorrhoeae* isolated from the 1,010 samples processed in this study are shown in Table 1. Both media were comparable in their ability to support the growth of gonococci isolated from clinical specimens, with a total of 187 gonococcal isolates being recovered on each of the media. Importantly, there were no isolates of *N. gonorrhoeae* which were recovered on only one of the media.

Figure 1 shows the comparative colonial growth on NYC and NYC-Hb media of *N. gonorrhoeae* isolated from a typical clinical sample after 24 h of incubation. Although the gonococcal colonies on the NYC medium were usually larger than those on the NYC-Hb medium, the relative numbers of colonies on each of the media were comparable. In addition, gonococcal growth was always detected on each of the media within the same incubation time period. For

example, 86% of the 187 gonococcal isolates recovered in this study were detected on both media within 24 h of incubation, and the remaining isolates were recovered after an additional day of incubation.

DISCUSSION

Faur and her associates (7) reported that hemoglobin in the form of lysed horse erythrocytes was a necessary ingredient in the original formulation of NYC medium for supporting rapid, uniform growth of freshly isolated strains of gonococci. The results of our study have shown that although the presence of hemoglobin in the form of lysed horse erythrocytes stimulates the growth of clinical isolates of *N. gonorrhoeae*, the absence of this component from NYC medium does not have a detrimental effect on the recovery of gonococci isolated from clinical specimens. This observation is in agreement with the findings of others (12, 13, 15, 17) who have reported the reliable recovery of gonococci on hemoglobin-free culture media.

NYC medium has also been reported by Faur and her colleagues (8) to be comparable to conventional methods in supporting the growth of urogenital mycoplasmas isolated from clinical specimens. In another study (5), these investigators showed that the recovery rate of these mycoplasmas was affected by the species of animal blood used in the medium. However, in that report it was unclear whether the recovery rate of urogenital mycoplasmas was dependent upon the species of animal erythrocytes or animal plasma used in the medium. It is conceivable that the differences in mycoplasma recovery rates were attributable to a particular species of animal plasma that may have contained specific or cross-reacting antibodies to urogenital mycoplasmas which could adversely affect their growth on the medium.

In the present study, we did not evaluate the effect that the absence of the hemoglobin component from NYC medium would have on the recovery of urogenital mycoplasmas. However, we showed in a previous report (10) that a modified formulation of NYC medium containing no lysed horse erythrocytes was capable of supporting the rapid growth of stock strains of *Mycoplasma pneumoniae*. As such, it is likely that urogenital mycoplasmas will grow on NYC-Hb medium as well.

The results of this study have shown that the preparation of NYC medium can be facilitated and that its cost can possibly be reduced by the elimination of lysed horse erythrocytes from the formulation without adverse effect on the recovery of *N. gonorrhoeae* isolated from clinical

TABLE 1. *Gonococcal recovery rates on NYC and NYC-Hb media*

Source	No. of specimens cultured	No. of gonococci recovered on both media ^a
Urethra	518	122 (23.6)
Cervix	197	51 (25.9)
Pharynx	230	7 (3.0)
Rectum	65	7 (10.8)
Total	1,010	187 (18.5)

^a Numbers in parentheses denote percent positive cultures.

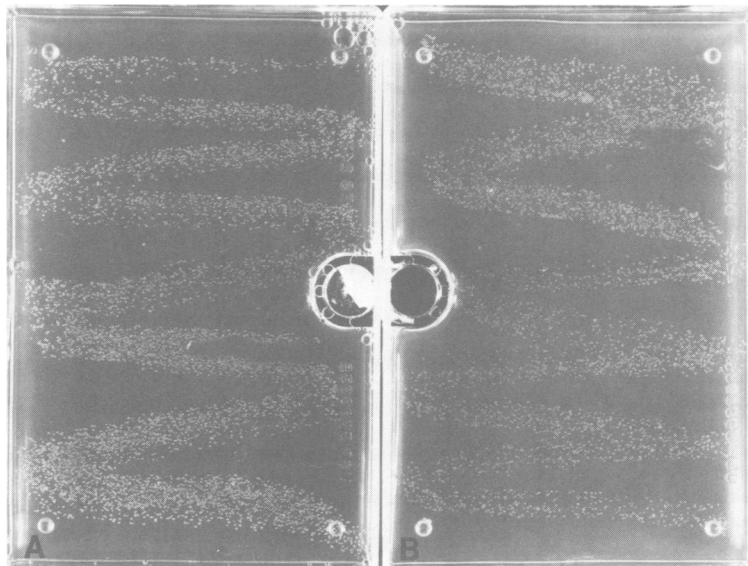


FIG. 1. Comparative growth on NYC (A) and NYC-Hb (B) media of *N. gonorrhoeae* isolated from a clinical specimen after 24 h of incubation.

specimens. The effect that this formulation change will have on the recovery of urogenital mycoplasmas requires additional evaluation.

ACKNOWLEDGMENTS

We thank Ralph Prowda and the nursing and clerical staffs of the Onondaga County Department of Health, Syracuse, N.Y., for their assistance in this study.

This study was supported in part by a grant from Scott Laboratories, Inc., Fiskeville, R.I.

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