

Simplified Media for Isolating *Neisseria gonorrhoeae*

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Two simplified media for isolating *Neisseria gonorrhoeae* are described. They differ from the modified Thayer-Martin medium in the enrichment and antibiotics used for preparation. The enrichment for one, enrichment 4 medium, contains only four ingredients, and amphotericin B is used instead of nystatin. This medium is comparable to the modified Thayer-Martin medium for isolating *N. gonorrhoeae*. It is convenient to prepare and is only one-third the cost of Thayer-Martin medium. It is a suitable alternative to the modified Thayer-Martin. The enrichment 5 medium is a hemoglobin-free version of enrichment 4 medium. It is somewhat more selective for contaminants but is also more inhibitory for *N. gonorrhoeae*.

An important contribution to the control of gonorrhea was made by the introduction of selective medium (4). Since then, many modifications have been made, and the modified Thayer-Martin (TM) medium (3) is an improvement over previous formulas. Our laboratory has been using a modified version of this medium (1.3% agar instead of 2.0%) for 3 years, and it has given satisfactory results. A major problem in using this medium has been the cost, and we describe below two simplified media that reduce the cost considerably.

MATERIALS AND METHODS

Preparation of media. (i) **E4 medium.** Enrichment 4 (E4) solution contained the following ingredients (per milliliter) in distilled water: L-cysteine HCl, 260 mg; L-glutamine, 100 mg; coenzyme 1, 4 mg; cocarboxylase, 2 mg (all from Sigma Chemical Co.). The solution was stored in 1-ml samples at -70°C . The enrichment has been kept at this temperature for 5 months without deterioration. Both E4 and enrichment 5 (E5) solutions are modifications of earlier formulations (5; C. E. Lankford, *Bacteriol. Proc.*, G40, p. 20, 1950).

Antibiotic solutions (i) and (ii) contained the following (per milliliter) in distilled water: (i) vancomycin (Eli Lilly & Co.), 3 mg; colistin methane sulfonate sodium (Banyu), 7.5 mg; trimethoprim lactate (Burroughs Wellcome), 5 mg; and (ii) amphotericin B (E. R. Squibb & Sons), 1 mg. The two antibiotic solutions were prepared separately, since a precipitate was formed when colistin methane sulfonate sodium was mixed with amphotericin B. The solutions were stored in 1-ml samples at -70°C .

The completed E4 medium contained the following (per liter) in distilled water: GC agar base (BBL), 36 g; agar (granulated, BBL), 3 g; hemoglobin (BBL), 10 g; E4 solution, 1 ml; antibiotic solution

(i) 1 ml; antibiotic solution (ii), 1 ml; dextrose solution (25%), 10 ml.

The GC agar base (with added agar) and hemoglobin solutions were prepared separately in 500 ml of distilled water. After autoclaving, they were cooled to 56°C and mixed. The premixed enrichment, antibiotic, and dextrose solutions were then added. The medium was then dispensed into petri dishes. The final pH of the medium is 7.2.

(ii) **E5 medium.** E5 medium was similar to the E4 medium, except that the hemoglobin and E4 solutions were replaced by 1 ml of E5 solution. This solution contained the same ingredients as E4 solution in addition to 5 mg of ferric nitrate per ml.

(iii) **Modified TM medium.** Modified TM medium was similar to the E4 medium, except that the enrichment and antibiotic solutions were replaced by 10 ml of IsoVitaleX, 10 ml of VCN (BBL), and 0.25 ml of trimethoprim lactate (20 mg/ml) for each liter of medium. The final concentration of trimethoprim lactate was 5 mg/liter of medium.

Evaluation of media. For the laboratory evaluation of media, 40 strains of recently isolated *Neisseria gonorrhoeae* were suspended in nutrient broth. These strains were identified by colonial morphology on modified TM medium, oxidase reaction, Gram stain microscopic examination, and sugar fermentations. The suspensions were streaked on the different media, and the plates were incubated at 36°C in a carbon dioxide atmosphere, using candle jars. The plates were examined after overnight and 48-h incubations. For the inoculum dilution test, 10-fold dilutions were made on 20 strain suspensions. A loopful of organisms was taken from each dilution and inoculated on the test media. The media were incubated as above. The maximum inoculum titer for which growth was detected on each medium was recorded. In the field trial, cervical and rectal swabs were taken from 597 patients attending a venereal disease clinic and inoculated on the different media. The order in which the media were inoculated was rotated every day. The plates were incubated as

above. The number of times *N. gonorrhoeae* or any contaminant was isolated on each plate was recorded. Media contamination was rated according to the number of plates that grew at least one colony of contaminant.

Vancomycin selectivity test. E4 and E5 media were prepared without adding the usual antibiotic. To 15-ml samples of each medium vancomycin was added to give the following final concentrations: 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 $\mu\text{g/ml}$. These were then poured into petri dishes and allowed to set. Suspensions in nutrient broth were prepared from four strains of *Staphylococcus epidermidis* isolated from clinical material. These were streaked on the media and incubated at 37°C overnight. The next day the minimal inhibitory concentrations of vancomycin for the bacteria were determined on the two media.

RESULTS

In the laboratory evaluation of media, the 40 strains of *N. gonorrhoeae* grew equally well on all three media tested. In the inoculum dilution test (Table 1), the E4 medium was comparable to the modified TM medium. The maximum inoculum titers for 18 strains were identical for both media. For one strain a few colonies were grown on E4 medium at a dilution of 10^{-7} , whereas on modified TM it grew at a dilution of 10^{-6} . On the other hand, another strain grew at 10^{-6} on modified TM but at 10^{-5} on E4 medium. The E5 medium was fractionally less sensitive than the modified TM. Two strains grew on it at one titer lower than on modified TM.

In the field trial, *N. gonorrhoeae* was isolated 78 times from E4 medium and 77 times from modified TM. The growth on both media was comparable (Table 2). On E5 medium *N. gonorrhoeae* was isolated 76 times, but it was noticed that on a number of occasions the colonies were smaller and fewer. E4 medium had slightly more contaminants (55%), that modified TM (52%), but E5 medium had the least contamination (34%). The contaminants were mostly gram-positive cocci.

The vancomycin selectivity test (Table 3) showed that the antibiotic was less effective in the presence of hemoglobin. The minimal inhibitory concentration of the antibiotic for the four *S. epidermidis* strains in the hemoglobin-

TABLE 1. Comparison of data for growth of 20 *N. gonorrhoeae* strains

Medium	No. of strains grown with maximum inoculum titer of:			
	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Modified TM	5	7	7	1
E4	5	8	5	2
E5	5	8	7	

TABLE 2. Comparison of results on cervical and rectal specimens taken from 597 women

Medium	No. of <i>N. gonorrhoeae</i> isolated	No. of contaminants isolated (%)
Modified TM	77	313 (52)
E4	78	329 (55)
E5	76	200 (34)

TABLE 3. Growth of four *S. epidermidis* strains on media containing vancomycin

Medium ^a	No. of strains growing at vancomycin concn						
	0	0.5	1.0	1.5	2.0	2.5	3.0
E4	4	4	4	4	0	0	0
E5	4	4	0	0	0	0	0

^a Media contained no antibiotics other than vancomycin in the amounts shown.

containing medium was 2.0 $\mu\text{g/ml}$, whereas in the hemoglobin-free medium it was 1.0 $\mu\text{g/ml}$.

DISCUSSION

The E4 solution differs from IsoVitaleX in containing only four ingredients instead of twelve. The concentrations of the labile coenzyme I and cocarboxylase were increased. Because of high solubility of these ingredients 1 liter of medium can be prepared with 1 ml of the enrichment. This made preparation and storage of the enrichment easy. The preparation of IsoVitaleX from its ingredients is tedious, and the final volume of enrichment is 10 ml/liter of medium. Simplified enrichments have been previously used for the preparation of nonselective media for the gonococcus (5; Lankford, *Bacteriol. Proc.*, G40, p. 20, 1950).

Amphotericin B has been chosen instead of nystatin because it has been shown to be a more effective antifungal agent (1). Although the antibiotic solutions are in two 1-ml samples per liter of medium, they occupy less storage space than the commercial VCN preparation. Because of the small volumes needed per liter of medium, both enrichment and antibiotic solutions may be conveniently lyophilized by laboratory freeze-dryers.

The E4 medium was as sensitive as the modified TM medium in supporting the growth of *N. gonorrhoeae* from laboratory cultures and patients. Contamination was slightly increased, but this did not affect the isolation rate from patients. The beneficial effect expected of amphotericin B (1) was not seen in this study. This was because the contaminants were mainly gram-positive cocci.

The E5 medium was found to be slightly more inhibitory than the modified TM medium. In the inoculum dilution test, two strains grew at one titer lower than with the modified TM. In the field trial, though the isolation rate was comparable, the colonies were occasionally smaller and fewer. The contamination on this medium was also less than that found on the hemoglobin-containing media. As the contaminants were mostly gram-positive cocci, it was felt that the hemoglobin might be binding some of the vancomycin. This was confirmed by the finding that in the presence of hemoglobin a higher concentration of vancomycin was required to inhibit the four strains of *S. epidermidis*. Hence, in the hemoglobin-free medium the efficacy of vancomycin was higher, and it inhibited more contaminants and some of the vancomycin-sensitive *N. gonorrhoeae*. In a study involving male patients (2), it was found that a hemoglobin-free medium (containing IsoVitaleX, vancomycin, colistin, and nystatin) also grew fewer contaminants than the TM medium. However, the isolation of *N. gonorrhoeae* was not adversely affected. This could be because the *N. gonorrhoeae* strains were more resistant to vancomycin than those in this study.

The cost of preparing the E4 medium is approximately one-third that of the modified TM medium. It is easy to prepare, and storage for the enrichment and antibiotic solutions takes up less space than the corresponding commercial preparations for the modified TM medium. It is a suitable alternative to the modified TM medium for isolating *N. gonorrhoeae* from pa-

tients. The presence of asymptomatic carriers of *N. gonorrhoeae* in both males and females is now well recognized. A comprehensive program to detect this vast reservoir of asymptomatic carriers might involve extending the screening program for gonorrhea to lower-risk groups. The availability of a low-cost medium, such as the E4 medium, should make this more feasible economically.

The E5 medium is even cheaper. It is also more convenient to produce, as there is no necessity to prepare the hemoglobin solution. Although the isolation rate for *N. gonorrhoeae* on the E5 medium is comparable to that on the modified TM, it is fractionally more inhibitory. Further study is being carried out to make it less inhibitory.

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